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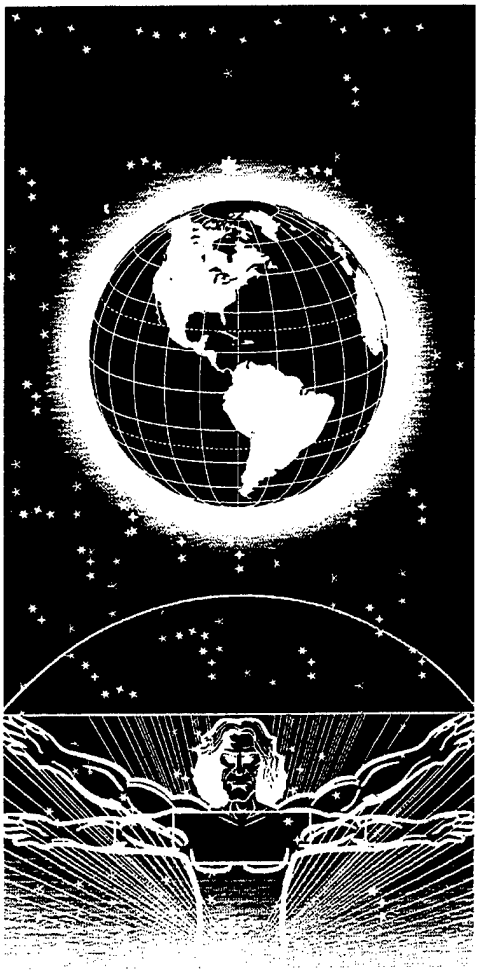

# UNITED STATES AIR FORCE RESEARCH LABORATORY

# ENDOCRINE DISRUPTORS: AN EVALUATION OF SOLVENTS, DEICERS AND JET FUELS

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**October 1997**



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This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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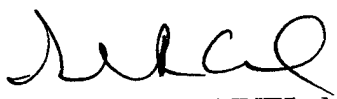

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Recent publications have drawn public attention to the growing evidence that a number of synthetic and naturally-occurring chemicals may disrupt normal function of endocrine systems. The scientific debate regarding endocrine disrupting chemicals (EDCs) centers around the insufficient data available to determine the ecological and human risks associated with environmental contaminants. This is partially due to the complex role of the endocrine system in regulating multiple physiological functions. Currently, the majority of EDC studies are focused on a few, well-known substances: DDT/DDE, dioxins and PCBs. Research fails to provide data on the majority of chemicals in use. The U.S. Air Force is placing emphasis on proactive reviews of its materials to preclude revelations which may impact mission readiness. The project evaluated the health effects of chemicals commonly used by the Air Force for evidence of potential endocrine disruptor activity. The chemicals selected for review were compounds not previously evaluated for endocrine disrupting activity: organic solvents (trichloroethylene, trichloroethane, dichloroethane, methyl ethyl ketone, methyl isobutyl ketone and perchloroethylene), deicing and anti-icing agents (potassium acetate, sodium acetate, ethylene glycol,urea, propylene glycol, sodium formate and calcium magnesium acetate) and jet fuels and related hydrocarbons (toluene, ethylbenzene, xylene, jet fuel and diesel).

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TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF APPENDICES

## PREFACE

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| µCi | micro-Curie |
| µg | microgram |
| µl | microliter |
| µM | micromolar or micromole/liter |
| µmol | micromole |
| 2,3,7,8-TCDD | 2,3,7,8-tetrachlorodibenzo-*p*-dioxin |
| 2,3,7,8-TCDF | 2,3,7,8-tetrachlorodibenzofuran |
| ACTH | adrenocorticotropic hormone |
| ADH | antidiuretic hormone |
| Ah | aryl hydrocarbon |
| AHH | aryl hydrocarbon hydroxylase |
| ALDH | aldehyde dehydrogenase |
| ALH | amplitude of lateral head displacement |
| ALP | alkaline phosphatase |
| AMP | adenosine monophosphate |
| AST | aspartate aminotransferase |
| ATP | adenosine 5'-triphosphate |
| BOD | biochemical oxygen demand |
| BPA | bisphenol A |
| BTEX | benzene, toluene, ethylbenzene and xylenes |
| BuChE | butyrlcholinesterase |
| $Ca^{2+}$ | calcium cation |
| CCK | cholecystokinin |
| cDNA | complimentary deoxyribonucleic acid |
| CENR | Committee on Environment and Natural Resources |
| CERC | chick embryo neural retina cells |
| CHEST | chick embryotoxicity screening test |
| CI | confidence interval |
| CIIT | Chemical Industry Institute of Toxicology |
| CMA | calcium magnesium acetate |
| CNS | central nervous system |
| $D_2$ | dopamine receptor |
| DCA | dichloroacetic acid |
| DCE | 1,2-dichloroethane |
| DDE | DDT degeneration product: 4,4'-dichlorodiphenyldichloroethylene |
| DDT | 4,4'-dichlorodiphenyl trichloroethane |
| DEHP | di-2-ethylhexyl phthalate |
| DES | diethylstilbestrol |
| DHT | dihydrotestosterone |
| dl | deciliter |
| DNA | deoxyribonucleic acid |
| DPN | diphosphopyridine nucleotide |
| $E_2$ | 17β-estradiol |
| $E_3$ | estradiol |
| $EC_{50}$ | concentration effective in 50% of tested population |
| EDC | endocrine disrupting chemical |
| EEG | electroencephalogram |
| EGME | ethylene glycol monomethyl ether |

| | |
|---|---|
| EMG | electromyogram |
| EPA | Environmental Protection Agency |
| ER | estrogen receptor |
| ESC | embryonic stem cell |
| $F_0$ | initial generation |
| $F_1$ | first generation, offspring of $F_0$ |
| $F_2$ | second generation, offspring of $F_1$ |
| $F_3$ | third generation, offspring of $F_2$ |
| FAA | Federal Aviation Administration |
| FETAX | frog embryo teratogenesis assay - *Xenopus* |
| FIFRA | Federal Insecticide, Fungicide and Rodenticide Act |
| FOIA | Freedom of Information Act |
| FSH | follicle stimulating hormone |
| g | gram |
| GC | gas chromatography |
| GH | growth hormone |
| GI | gastrointestinal |
| hCG | human chorionic gonadotropin |
| hER | human estrogen receptor |
| IARC | International Agency for Research on Cancer |
| $IC_{50}$ | concentration inhibitory to 50% of tested population |
| IU | international unit |
| IUGR | intrauterine growth retardation |
| kg | kilogram |
| l | liter |
| $LD_{10}$ | dose lethal to 10% of tested population |
| $LD_{50}$ | dose lethal to 50% of tested population |
| LDH | lactate dehydrogenase |
| LH | luteinizing hormone |
| LHRH | luteinizing hormone releasing hormone |
| LIF | leukemia inhibiting factor |
| LOAEL | lowest observable adverse effect level |
| LOEC | lowest observed effect concentration |
| LOEL | lowest observed effect level |
| M | molar or moles/liter |
| *m-* | *meta-* |
| $m^3$ | cubic meter |
| mCi | milli-Curie |
| MCL | maximum contaminant level |
| MEC | minimal effective concentration |
| MEK | methyl ethyl ketone |
| MFO | mixed function oxidase |
| mg | milligram |
| MIBK | methyl isobutyl ketone |
| mIU | milli-international unit |
| ml | milliliter |
| mM | millimolar or millimoles/liter |
| mm | millimeter |
| mmol | millimole |
| mol | mole |
| mRNA | messenger ribonucleic acid |
| NADP | nicotinamide adenine dinucleotide phosphate |
| ng | nanogram |
| NIEHS | National Institute of Environmental Health Sciences |

| | |
|---|---|
| NIOSH | National Institute of Occupational Safety and Health |
| nl | nanoliter |
| NOAEL | no observable adverse effect level |
| NOEL | no observable effect level |
| NTP | National Toxicology Program |
| *o-* | *ortho-* |
| OEC | oviduct epithelial cells |
| OSHA | Occupational Safety and Health Administration |
| p | probability |
| *p-* | *para-* |
| PAH | polycyclic aromatic hydrocarbon |
| PBB | polychlorinated biphenyl |
| PCB | polychlorinated biphenyl |
| PCE | perchloroethylene or tetrachloroethylene |
| PNS | peripheral nervous system |
| $PO_4$ | phosphate anion |
| ppm | parts per million |
| PTH | parathyroid hormone |
| PVC | polyvinyl chloride |
| QSAR | quantitative structure activity relationship |
| r | correlation coefficient |
| RIDS | runway ice detection systems |
| SGPT | serum glutamic pyruvic transaminase |
| SHA | sperm head abnormality |
| SHBG | sex hormone binding globulin |
| SMR | standardized mortality ratio |
| STH | somatotropin |
| $T_3$ | triiodothyronine |
| $T_4$ | thyroxine |
| TCA | trichloroacetic acid |
| TCE | trichloroethylene |
| TCOH | trichloroethanol |
| TLV | threshold limit value |
| TMA | tetramethylammonium chloride |
| TRH | thyrotropin releasing hormone |
| TRI | 1,1,1-trichloroethane |
| TWA | time weighted average |
| v/v | volume to volume ratio |
| w/w | weight to weight ratio |
| WHO | World Health Organization |
| WWII | World War II |
| YES | yeast estrogen system |

# ENDOCRINE DISRUPTORS: AN EVALUATION OF SOLVENTS, DEICERS AND JET FUELS FOR ENDOCRINE ACTIVITY

## INTRODUCTION

The recently published book, *Our Stolen Future* (Colborn *et al.*, 1996) has drawn attention to an environmental and occupational issue that has concerned the U.S. Environmental Protection Agency (EPA) and others for some time, the growing evidence that a number of synthetic and naturally occurring chemicals may disrupt the normal functioning of endocrine systems of wildlife and humans. Reports listing chemicals with potential reproductive and/or endocrine disrupting effects have been recently generated by several agencies and public interest groups including: U.S. EPA, World Wildlife Fund, National Wildlife Federation, California EPA and Illinois EPA. The state of Ohio has included reproductive and endocrine disrupting chemicals in their toxic release inventory. The federal government has responded to this issue by coordinating a Work Group on Endocrine Disruptors under the auspices of the Committee on Environment and Natural Resources (CENR).

The scientific debate regarding endocrine disrupting chemicals (EDCs) centers around the insufficient data available to determine the ecological and human risks associated with environmental contaminants. This is due, in part, to the complex role of the endocrine system in regulating multiple physiological functions and the difficulty in determining whether the effects are the result of primary disturbances of endocrine function or secondary effects (i.e., are other systems in the body more sensitive to exposure to these chemicals and the endocrine system is affected as a consequence?). Also, given the fact that there are other pressing public health concerns with less associated uncertainty (such as habitat destruction, global warming, poor air quality or drinking water disinfection), it is difficult to rank it as a top environmental and public health priority.

Much of the attention has focused on chemicals that affect female and male sex hormones, because certain human cancers that are influenced by hormones, such as those of the breast, prostate and testes, appear to be on the rise. Attention has also focused on the potential impact of certain chemicals on reproduction and development. The first widespread recognition of the potential for toxicants to cause reproductive and developmental effects came in the 1960s. This was due to recognition of the association of thalidomide with unusual birth defects in children exposed during a narrow time span in early pregnancy. Since then, and especially in the last decade, increasing concern has been expressed about effects of environmental and occupational exposures on reproduction. The concern has intensified due to the increase in women in the workplace. For men, the effects of workplace exposures was recognized in the late 1970s, when male infertility was associated with exposure to a pesticide (Whorton *et al.*, 1977).

Colborn suggests that historical perspective on widespread exposure to xenobiotics provides insight to troubled wildlife populations (e.g., large-scale mortality among dolphins, porpoise, seal and whale populations that began in 1987 (Kuehl *et al.*, 1991)) and reduced fertility in the human population. Table 1 provides a chronology of world-wide chemical exposure events.

Polychlorinated biphenyls (PCBs) were introduced in 1929 and DDT followed in 1938. During the 1940's an onslaught of chemicals were introduced. By the mid 1960's, the individual exposed since the 1940's began to bear children who were the first generation exposed *in utero.* This second generation reached reproductive age in about 1980. A meta-analysis that reexamined 61 sperm-count studies revealed that worldwide sperm count has decreased by approximately 50% since 1938 (Carlsen *et al.,* 1992).

TABLE 1: CHRONOLOGICAL EXAMINATION OF HUMAN EXPOSURE TO SYNTHETIC CHEMICALS

| Time Span | Exposure Event |
|---|---|
| 1929 | PCBs introduced |
| 1938 | DDT first manufactured |
| 1940s-WWII | First wide scale exposure to man-made chemicals |
| 1940s-1950s | First generation exposed postnatally |
| 1950s-1970s | First generation born that was exposed in womb |
| 1970s-1990s | First generation exposed in womb reaching reproductive age |

Adapted from Colborn (1994).

## OBJECTIVES

Currently, the majority of research reported on EDCs is focused on a few, well-studied substances. The Bureau of National Affairs, Inc. reported in the November, 1996 issue of *Chemical Regulation Reporter* that approximately 71 percent of the ongoing federal research is focused on DDT/DDE, dioxins and PCBs. While those substances are strongly suspected of disrupting hormone systems, the research fails to provide data on the majority of chemicals in use. In July 1991 at Racine, Wisconsin, a multidisciplinary group of experts gathered to assess what is known about endocrine disruptors. Chemicals identified to disrupt the endocrine system include: DDT and its degradation products, di-2-ethylhexyl phthalate (DEHP), dicofol, hexachlorobenzene, kelthane, kepone, lindane and other hexachlorocyclohexane congeners, methoxychlor, octachlorostyrene, synthetic pyrethroids, triazine herbicides, certain fungicides, some PCB congeners, 2,3,7,8-TCDD and other dioxins, 2,3,7,8-TCDF and other furans, cadmium, lead, mercury, tributyltin and other organo-tin compounds, alkyl phenols (non-biodegradable detergents and anti-oxidants present in modified polystyrene and PVCs), styrene dimers and trimers, soy products and laboratory animal and pet food products (Colborn *et al.*, 1996).

The objectives of this project were: a) to review the health effects from a select list of chemicals commonly used by the U.S. Air Force, and b) to evaluate those health effects for evidence, but not necessarily overwhelming proof, of potential endocrine disruptor activity. The military is placing emphasis on proactive reviews of its common hazardous materials to preclude revelations which may impact their mission readiness. Therefore, to broaden the knowledge of potential EDCs, the chemicals selected for review in this paper were chemicals which have not been evaluated for endocrine disrupting activity. They included the following: organic solvents (trichloroethylene, trichloroethane, dichloroethane, methyl ethyl ketone, methyl isobutyl ketone and perchloroethylene), deicing and anti-icing agents (potassium acetate, sodium acetate,

ethylene glycol, urea, propylene glycol, sodium formate and calcium magnesium acetate) and jet fuels and related hydrocarbons (toluene, ethylbenzene, xylene, jet fuel and diesel).

Due to the enormous range of functions of the endocrine system, the focus of this paper was broad and the quality of each study reviewed was not necessarily addressed. The literature review was extensive but not exhaustive. A critical evaluation of all published data on every Air Force chemical and how those chemicals affect endocrine activities is not within the scope of this review. The following paragraphs describe the types of studies searched and reviewed; however, the majority of studies available were developmental toxicity assays.

Epidemiological/occupational studies on fertility and reproductive outcome were reviewed. Reproductive disorders are important health problems and affect the quality of life that people expect. It is estimated that 10-15% of all married couples or couples living together have experienced an infertility problem (Baird and Wilcox, 1986). Epidemiological studies are useful for validation of experimental data. However, the disadvantage of epidemiological studies for identifying reproductive disorders and hence, potential endocrine disruptors, is that these studies are less sensitive than studies focusing on particular parameters such as hormonal imbalances or changes in semen quality. There are many extraneous factors that have the potential to confound studies of infertility or pregnancy outcomes and ideally should be controlled for in the design and/or analysis of the investigations. Such potential confounding factors are 1) sexually transmitted infections, the determinants of which are thought to be the number of sexual partners and the usage and type of contraception, and 2) sexual activity, taking into consideration both timing and frequency of intercourse. These two factors are probably the major confounders, but other factors must also be considered such as exposure of spouse to different agents, lifestyle factors, small sample sizes and lack of appropriately matched controls.

Bioassays (both *in vivo* and *in vitro*) on the chemicals of concern were extensively reviewed for this project. These tests included toxicity assays, reproductive and developmental assays (including multigenerational tests) and screening tests, to name a few. Information on quick *in vitro* screening tests was limited because these assays are currently being developed. Therefore, much of the literature discussing validation of newer screening assays has apparently not been published at this point.

Although wildlife population surveys may be helpful in identifying endocrine disruptor chemicals in the environment, such studies dealing with exposure to the chemicals mentioned were not searched and reviewed for this project. This review focused on studies indicative of possible human effects. Wildlife population studies provide a good source of hypothesis generation regarding environmental hazards; however, inclusion of these studies was beyond the scope of this project.

## APPROACH

Because the initial focus of this project (to address the major chemicals of concern at Air Force sites and define research needs, gaps and uncertainty in risk assessment pertaining to endocrine disruption) was so broad, it was necessary to limit the number of chemicals assessed. Therefore lists of commonly used military hazardous materials were reviewed in light of EDC lists provided in the scientific literature and by several federal agencies, including

Colborn's initial list of about 40 suspected EDCs. Appendices A through C contain lists of suspected EDCs generated from initial literature searches; the reported dose levels and associated effects were included when available. Hazardous materials, which have been assessed as to their endocrine activity elsewhere (e.g., PCBs, dioxins and furans, pesticides and alkyl phenols), were excluded. The final list was limited to a few chemical classes used extensively at Air Force sites. As mentioned above, the general classes of chemicals selected were solvents, deicers and jet fuels.

The literature search was performed on the National Library of Medicine's Medline (1966 to present) and Toxline (pre-1981 to present) databases. The terms featured in Table 2 were crossed with the identified chemicals of concern. Articles with information concerning those topics were retrieved from local libraries, where available. It should be noted that only a few neuroendocrine-related terms were used as search words, producing a potential data gap in this review.

TABLE 2: ENDOCRINE DISRUPTOR SEARCH TERMS

| | | |
|---|---|---|
| androgen (ic) | estrus cycle | pituitary |
| androstenedione | fertility | placenta |
| biomarker | fertilization | progesterone |
| bone development | fetus | prolactin |
| cancer: breast, ovarian, uterine, prostate, testicular | follicle stimulating hormone (FSH) | receptor (hormone) |
| developing embryo | gestation | Sertoli cell |
| developing fetus | hypothalamus | sexual behavior |
| developmental | implantation | sexual function (dysfunction) |
| embryo | infertility | sperm |
| endocrine | Leydig cell | teratology (teratogen) |
| epididymis | luteinizing hormone (LH) | testis |
| estradiol | MCF-7 cells | testosterone |
| estrogen (ic) | ovary (ovarian) | thyroid ($T_3$, $T_4$, TSH) |
| | | uterus |

Despite the growing scientific understanding of the effects of EDCs, there remain considerable uncertainties. One of the most significant data gaps is the actual mode of action by which xenobiotics with endocrine disrupting potential affect developmental and central nervous system (CNS) processes. Hence a brief review of endocrine function and potential mechanisms of disruption was included in this review.

The results of the literature searches revealed that a very limited amount of information has been published regarding screening techniques for EDCs. A complete screening battery has not necessarily been performed on the selected chemicals of concern. Screening techniques found in the literature, which have either been validated or are in the process of validation, were identified and described.

## GENERAL REVIEW OF ENDOCRINE FUNCTIONS

The endocrine system, together with the nervous system, acts as the body's communication network. The endocrine glands are composed of specialized endocrine cells which secrete hormones (chemical messengers) that travel through the bloodstream to influence target cells widely distributed in the body. There are five general functions to the endocrine system: 1) differentiation of the reproductive and central nervous systems in the developing fetus, 2) stimulation of sequential growth and development during childhood and adolescence, 3) coordination of the male and female reproductive systems, 4) maintenance of an optimal internal environment throughout the lifespan and 5) initiation of corrective and adaptive responses when emergency demands occur (Gray, 1990). Examples of neurotransmitters and hormones are listed in Table 3.

TABLE 3: EXAMPLES OF NEUROTRANSMITTERS AND HORMONES

| Chemical class | Hormone | Major Source | Major Effect |
|---|---|---|---|
| Amines | Dopamine | CNS | |
| | Norepinephrine | CNS, adrenal medulla | excitatory & inhibitory transmitter in CNS & PNS |
| | Epinephrine | Adrenal medulla | |
| Iodothyronines | Thyroxine ($T_4$) | Thyroid gland | increase in metabolic activity in most cells |
| | Triiodothyronine ($T_3$) | Peripheral tissues (conversion site of $T_3$ to $T_4$) | same effect as thyroxine but more rapid |
| Small peptides | Vasopressin (antidiuretic hormone; ADH) | Posterior pituitary | elevation of blood pressure by constriction of small blood vessels; increase in water resorption in kidney tubules |
| | Oxytocin | Posterior pituitary | stimulate uterine contraction & lactation; may have a role in sperm motility |
| | Thyrotrophin-releasing hormone (TRH) | Hypothalamus, CNS | stimulation of anterior pituitary to secrete luteinizing hormone (LH) |

| Chemical class | Hormone | Major Source | Major Effect |
|---|---|---|---|
| Small peptides (continued) | Gonadotropin | Hypothalamus, CNS | promotes gonadal growth and function |
| | Somatostatin | Hypothalamus, CNS | inhibition of somatotropin release from anterior pituitary |
| Proteins | Insulin | Beta cells of pancreatic islets | utilization of carbohydrates; stimulation of protein synthesis; stimulation of lipid synthesis in fat cells |
| | Glucagon | Alpha cells of pancreatic islets | insulin antagonist; increases blood glucose |
| | Growth hormone or somatotropin (GH, STH) | Anterior pituitary | stimulates cell division |
| | Parathyroid hormone (PTH) | Anterior pituitary | increase in bone resorption, thereby increasing blood $Ca^{2+}$ & $PO_4$ |
| | Adrenocorticotropic hormone (ACTH) | Parathyroid gland | stimulation of adrenal cortex to produce cortisol; fatty acid release from fat cells |
| | Secretin | Anterior pituitary | incites secretion of pancreatic juice |
| | Cholecystokinin (CCK) | Gastrointestinal (GI) tract | stimulates contraction of the gall bladder |
| | Gastrin | GI tract | stimulates secretion of HCl upon contact with gastric contents |
| Glycoproteins | Follicle-stimulating hormone (FSH) | Anterior pituitary | stimulation of ovarian follicles to grow & secrete estradiol; stimulation of spermatogenesis in testis |

| Chemical class | Hormone | Major Source | Major Effect |
|---|---|---|---|
| Glycoproteins (continued) | Luteinizing hormone (LH) | Anterior pituitary | stimulates oocyte maturation & ovulation and progesterone secretion from ovary; stimulates testes to produce testosterone |
| | Thyroid-stimulating hormone (TSH) | Anterior pituitary | stimulates thyroid to produce thyroxine; fatty acid release from fat cells |
| Steroids (fat soluble) | Estrogens ($E_2$, $E_3$) | Ovary, placenta | development and maintenance of secondary female sex characteristics; maturation and cyclic function of accessory sex organs; development of duct system in mammary glands |
| | Progesterone | Corpus luteum, placenta | anti-estrogenic, regulation of menstrual cycle |
| | Testosterone | Testis | development & maintenance of secondary male sex characteristics; maturation & normal function of accessory sex organs |
| | Dihydrotestosterone (DHT) | Testosterone sensitive tissues | same actions as testosterone |
| | Glucocorticoids | Adrenal cortex | aids metabolism |
| | Aldosterone | Adrenal cortex | facilitates potassium exchange for sodium in distal tubules |

Adapted from Chattoraj and Watts, 1987 and Gray, 1990.

## Examples of Mechanisms by which Compounds Disrupt Endocrine Function

Hormones are greatly diluted in the bloodstream and therefore must act at very low concentrations (typically less than $10^{-8}$ M) (Alberts *et al.*, 1983). It is not surprising that extremely low concentrations of endocrine-like chemicals may be disruptive to the endocrine system. It has been found recently that only one, very low dose of dioxin administered during gestation can change the sexual development of rat offspring (Mably *et al.*, 1991).

The mechanisms by which EDCs have their impact vary, but they share the general properties of: 1) mimicking the effects of natural hormones by recognizing their binding sites, 2) antagonizing the effect of these hormones by blocking their interaction with physiological binding sites, 3) reacting directly and indirectly with the hormone in question, 4) altering the natural pattern of hormone synthesis or 5) altering hormone receptor levels. Some compounds disrupt endocrine function through more than one of the above mechanisms (Alberts *et al.*, 1983).

Any disruption of the endocrine system during development may permanently alter that organism. Typically these effects are irreversible. For example, many sex-related characteristics are determined hormonally during a window of time in the early stages of development and can be influenced by small changes in hormone balance. Evidence suggests that sex-related characteristics, once imprinted, may be irreversible. Estimating the incidence of these effects is difficult and determining the cause is even more complex because of the many confounding factors that must be considered. These factors include, but are not limited to: incomplete information concerning dose, timing and duration of exposure; unknown interactions between causes; difficulty in obtaining specimens; inadequacy of analytical techniques; the large number of potential causes of abortion and variations in individual susceptibility due to differences in genotype.

### Disruption of Testicular Function

There are several mechanisms of testicular malfunction. One mechanism is through the inhibition of FSH secretion. Some believe that estrogen-like compounds are involved in the fall in sperm count over the past 30 to 50 years. The mechanism is suggested to involve inhibition of FSH secretion and subsequent reduction of Sertoli cell multiplication during prenatal and prepubertal life. Because the numbers of Sertoli cells determine testicular size and sperm count in adulthood, the lower number of Sertoli cells will be the ceiling for sperm output. The effects of diethylstilbestrol (DES) were seen in prenatally exposed men whose semen show markedly poor quality (Lahdetie, 1995).

### Disruption of Pregnancy Physiology

Fertilization of the ovum by the sperm occurs in the fallopian tube after which it takes three days to reach the uterus and another four to five days before implantation in the wall of the uterus occurs. At that point a hollow sphere of cells, called the blastocyst, is formed during the first week. Injury to the cells at this point does not result in a specific developmental defect because the cells are not differentiated. However, at sufficiently high doses, death of the blastocyst can occur (Shane, 1989).

By the end of week two, blood flow through the embryo is established and embryonic differentiation begins the third week, when the cells are segregated into the three embryonic germ layers, referred to as endoderm, mesoderm, and ectoderm. Each germ layer gives rise to specific groups of primordial cells during weeks four through eight. This is known as organogenesis; the differentiated cells have more specialized metabolic requirements and are therefore more vulnerable to damage by adverse influences. Upon the completion of organ formation at the end of the twelfth week, the induction of major structural defects is no longer a factor of concern (Shane, 1989).

From the third month to birth is known as the fetal period. Continued tissue differentiation occurs and is closely associated with the development of the functional activity of the fetal organs. Insults during this stage result in microscopic structural defects and possible functional abnormalities (Shane, 1989). Because structural and functional maturation continues after birth in many organ systems such as the immune, nervous, hepatic, renal and endocrine systems, there is a growing concern about the possible adverse effects of environmental factors during infancy and childhood.

The outcome of pregnancy following exposure to a chemical depends upon the length of exposure, the stage of fetal development at the time of exposure, the magnitude of exposure and the nature of the chemical substance. The total dose that reaches the fetus is dependent on several factors: the magnitude of the dose, the exposure route, the rate of absorption by maternal system and the effectiveness of maternal homeostatic devices (i.e., detoxification, excretion or storage) (Shane, 1989).

Disruption of Sexual Differentiation

During sexual differentiation (occurring during the first trimester of human pregnancy), there are critical periods when the reproductive system is susceptible to chemically-induced disturbances that may result in irreversible effects such as infertility. A similar exposure during adulthood may only have a minor, reversible effect. Many of the effects from neonatal and fetal exposures do not manifest until after puberty.

The basic mechanisms underlying sexual differentiation are similar in all mammals although the timing of certain events differ. This suggests that chemicals which adversely effect reproductive development in rodents may be potential reproductive toxicants in humans as well. Developmental toxicants may act as hormone agonists by binding to and activating steroid hormone receptors (e.g., DES and DDT) or by competitively binding to and inhibiting receptors. Other chemicals may disrupt the endocrine system by altering hormone levels through inhibition of steroid hormone synthesis (e.g., alcohol) or stimulation of steroid hormone catabolism. Chemicals that inhibit mRNA translation or transcription processes during critical periods of development can also alter sexual differentiation (Colborn *et al.*, 1996). Sexual differentiation in the CNS is dependent upon hormone-induced changes in neurotransmitters because cells read the concentrations of neurotransmitters to determine their position during sex organ formation.

Another potential mechanism resulting in retarded fetal growth is by inhibiting acetylcholine synthesis, or the acetylcholine receptor, which in turn blocks amino acid uptake (Witorsch, 1995). The fact that the placenta synthesizes and releases acetylcholine may mean that the cholinergic system regulates amino acid uptake and transport in the placenta. A major factor

contributing to environmentally-induced intrauterine growth retardation (IUGR) is through a common blockade of placental amino acid transport.

## Disruption of Central Nervous System Development

The developing CNS is the organ system most frequently observed to exhibit congenital abnormalities. There are several ways things might go wrong in CNS development due to environmental exposure. An agent could interfere with cell proliferation, so that too few cells are produced. An agent could interfere with migration, causing cells to end up in the wrong place. An agent could interfere with the outgrowth of extensions from cells, with the establishment of synaptic connections or with the development of dozens of other properties of neurons that are needed to achieve the normal function of transmitting and receiving information. There are vulnerable periods during development. When cells are forming, agents which interfere with cell proliferation can cause damage. When neurons are making connections, agents which interfere with synaptogenesis can cause damage and so forth (Rodier, 1994). The following paragraph describe some of those vulnerable periods.

### Cell Production

The CNS developmental process that the most is known about is cell proliferation. Purkinje cells form in mid-gestation in the rodent; the smaller cells form mostly after birth. The large cells of the hippocampus form at that time and the dentate gyrus cells much later (Rodier, 1994). Luteinizing hormone releasing hormone (LHRH) producing cells of the hypothalamus form a little earlier. The LHRH cells control the release of pituitary hormones which stimulate and maintain the ovaries and testes. Interference with cell division on day 12, when LHRH cells are forming, would cause the animals have fewer cells expressing LHRH as adults. In addition, the injured animals would also have immature gonads and would go through puberty later than controls (Rodier, 1994).

The CNS has another vulnerability with regard to cell proliferation. It has no ability to replace missing neurons when it is mature, as some other tissues do. Experiments in which proliferation was interrupted for brief periods show that once the normal period of production of a particular cell type is over, any compensation generated by the developing tissue tends to enhance the numbers of the next cells on the production schedule, rather than the ones missing (Rodier, 1994).

### Cell Migration

Because neurons depend on physical contact between cells, misplacement of cells can cause problems. Methyl mercury is known to cause migration failure. Because human neurons are still migrating long after birth, the length of time required for its development makes the CNS subject to many injuries (Rodier, 1994).

Cell Differentiation

Thyroid hormones have been shown to act on neurological development by increasing the rate of neuronal proliferation in the cerebellum, acting as the "time clock" to end neuronal proliferation and stimulate differentiation. Once neurons are formed, they follow an orderly pattern of migration to the appropriate areas in the brain. A deficiency of thyroid hormones in the neonatal rat has shown disorganization of the cerebellar cortex (Porterfield, 1994). Exposure to hypothyroidism *in utero* can result in neurons which fail to extend enough processes to make appropriate connections. Myelinization is also delayed. Children born to hypothyroid mothers have shown brain dysfunction and a higher incidence of behavioral problems (Porterfield, 1994). Many environmental contaminants alter thyroid function, either inhibiting the thyroidal system or mimicking it. For example, PCBs and dioxin are similar structurally to $T_4$ and $T_3$, active thyroid hormones. In addition, they all have similar protein binding characteristics and bind to the same cytosolic aryl hydrocarbon (Ah) receptor and to thyroid hormone binding proteins such as transthyretin and the thyroid hormone receptor. These toxins act as weak agonists and block the action of thyroid hormones. In fact, dioxins can stimulate expression of v-erb-A, the gene encoding the putative thyroid hormone receptor. Consequently, the possibility exists that these toxins could, at low levels, be altering neurological development via their action on thyroid hormone availability during critical brain developmental periods (Porterfield, 1994).

Disruption of Metabolic Activity

Chemicals may disrupt endocrine functions indirectly, requiring metabolic activation to a toxic metabolite. This category includes polycyclic aromatic hydrocarbons (PAHs). Toxicants may also produce changes in the physiologic control mechanism involved in the regulation of the endocrine system. Changes in the control mechanisms may be brought about by modification of enzymes involved in steroid secretion or clearance. Examples of this include insecticides and the halogenated hydrocarbons: polybrominated biphenyls (PBBs), PCBs and DDT (Mattison, 1983).

Disruption of the Immune System

Hormones of the neuroendocrine system affect components of the immune system and mediators produced by immune components regulate the neuroendocrine response. The adrenal cortex is directly innervated by the sympathetic nervous system and releases catecholamines (primarily epinephrine and norepinephrine). The adrenal cortex is also stimulated through hormones of the hypothalamus and anterior pituitary, causing release of cortisol and aldosterone. Cortisol secretion leads to hyperglycemia and immune suppression. It enhances and prolongs the elevation of blood glucose levels initially achieved by epinephrine and norepinephrine. Therefore cortisol is essential for an effective response to stress (McCance and Huether, 1990).

Increased cortisol secretion is also associated with suppression of the immune system. This has a beneficial effect during stressful times by stabilizing the immune response (e.g., hyperinflammatory responses that destroy tissue or cause too much vasodilatation). But

chronic elevated cortisol levels cause severe suppression of the immune response (McCance and Huether, 1990).

Evidence of an increased rate of autoimmunity associated with prenatal DES exposure suggests the possibility that EDCs may induce a similar effect. Alteration of sex-steroid balance has been shown to lead to increased or accelerated onset of autoimmune syndromes in mice. In rats and mice, heavy metals such as lead, mercury and gold enhance autoimmune syndromes (Kavlock *et al.*, 1996).

## NATURALLY OCCURRING ENDOCRINE DISRUPTORS

Chemicals may act directly due to structural similarity to an endogenous compound (estrogen). These chemicals bind to the estrogen receptor and show estrogenic or anti-estrogenic activity. This category includes xenobiotics that bind to steroid hormonal receptors at high doses, e.g., DDT and other organochlorine pesticides, PCBs and PBBs (Scialli and Zinaman, 1993). Numerous edible plants with recognized estrogenically active compounds, called phytoestrogens, have been identified (Table 4).

TABLE 4: EDIBLE PLANTS WITH RECOGNIZED ESTROGEN ACTIVE COMPOUNDS

| Estrogens | Isoflavones | Coumestans | Resorcylic acid Lactones | Others |
|---|---|---|---|---|
| Licorice | Soybean | Alfalfa | Oats | Fennel |
| French bean | Chick-pea | Soybean sprouts | Barley | Carrot |
| Date palm | Cherry | Cowpea | Rye | Anise |
| Pomegranate | | Green beans | Sesame | Hops |
| Apple | | Red beans | Wheat | |
| | | Split peas | Peas | |

Adapted from Scialli and Zinaman, 1993

One class of phytoestrogens, the isoflavones, has received attention as beneficial to health. It is argued that isoflavones may prevent hormone-dependent diseases later in life (Setchell, 1985). An abundance of isoflavones is found in soybeans.

Dietary intake of phytoestrogens has yet to be associated with disrupted reproduction in humans. In sheep, however, grazing in pastures rich in a clover that contains a weak estrogen leads to infertility known as clover disease (Setchell, 1985).

## SCREENING TECHNIQUES FOR REPRODUCTIVE/DEVELOPMENTAL TOXICITY AND ESTROGENIC ACTIVITY

Current toxicity testing cannot keep pace with the rate of development of new chemicals. As a result there is an urgent need for new, inexpensive and quick toxicological tests. In recent years, some new screening tests for reproductive, developmental and EDC toxicity have been

developed. Screening tests tend to be more limited in scope than conventional tests. Data from screening assays are used to indicate a toxic potential of a chemical as either low priority or high priority needing further evaluation.

The EPA has formed an advisory panel to come up with *in vitro* tests to screen for environmental estrogens that pose the greatest potential threat. In August, 1996, Congress amended three environmental statutes that would require EPA to begin screening such chemicals within two years. Specifically, amendments were passed to the Safe Drinking Water Act, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Federal Food, Drug and Cosmetic Act mandating that: 1) EPA develop within two years a testing program to determine whether EDCs have an adverse effect on the endocrine systems of humans or wildlife and 2) companies that import, manufacture or register suspected EDCs must implement EPA's testing procedures and provide their data to EPA within three years (Hill, 1996).

## *In Vivo* Screening

There are several *in vivo* screening tests available. In human risk assessments, multigenerational tests are, at least, required for pesticides intended for food use; this enables an indirect assessment of latent endpoints and usually would entail exposure of animals during potentially sensitive life stages. The multigeneration testing protocols for human health effects used by the U.S. EPA have recently been modified to be more reflective of potential endocrine disruption, including aspects of female cyclicity, semen quality and hormonally sensitive organs pathology. However, even for human assessments, multigenerational tests are not used routinely enough to detect possible EDCs other than for food-use pesticides. The following are descriptions of two *in vivo* screening tests for teratogenicity and reproductive effects (Kavlock *et al.*, 1996).

### *In Vivo* Teratology Screening Test

This test is an alternative to conventional teratogenicity tests. The hypothesis is that most prenatal effects do not just produce specific defects but also are manifested in the postnatal period as a lack of viability and reduced growth. Pregnant mice or rats are exposed to a test substance from days 8 through 12 of the pregnancy. A control group is not exposed. One dose level is used (minimum toxic dose for a mother animal). The mothers are weighed during the period of exposure. After birth, the litter is weighed on the first and third days. Stillborn young and young that die after birth are dissected and examined for defects. The focus is on malformations as endpoints (Kavlock *et al.*, 1996).

### Preliminary Reproduction Toxicity Screening Test

The Preliminary Reproduction Toxicity Screening Test, another example of *in vivo* screening, is a reduced one-generation study which has been validated with several chemicals including ethylene glycol monomethyl ether (EGME) (Toppari *et al.*, 1996). The purpose of the test is to generate limited information concerning the effects of a chemical on male and female reproductivity. Measurements include gonadal function, mating behavior, conception, development of fetus and birth. Animals are dosed two weeks prior to mating and dosing

continues until the end of the study on postnatal day 4. The number of animals per group (including a control group) is generally about ten of each sex (expected to provide at least eight pregnant females per group). Effects on fertility and birth are registered. Live pups are counted and sexed and litters weighed on days 1 and 4 postpartum. Evaluation parameters include, among others, a detailed histological examination on the ovaries, testes and epididymides of at least the highest dose and control animals. The results of several one generation studies performed on the chemicals of concern are discussed in this paper.

## *In Vitro* Screening

Many *in vitro* test systems have been proposed as alternatives to whole animal testing for developmental toxicity or to detect certain endocrine disruptor activity. These tests are not able to replace animal testing, but can reduce the number of chemicals to be tested with live animals. *In vitro* techniques may also prove useful in the screening of complex chemical mixtures (e.g., in product development prescreening or in the screening of closely related chemicals). In addition, *in vitro* tests can be used as a tool for pinpointing the mechanisms underlying a known embryo-fetotoxic effect and as such provide information that can improve the interpretation of the results and consequently the extrapolation for laboratory animal experiments to humans. Tests include: the MCF-7 human breast adenocarcinoma cell proliferation assay for estrogen and other receptors; competitive receptor binding assays for estrogens, androgens and progestins; transfected cell assays for hormonal and antihormonal activity and *in vitro* alterations of whole and minced adrenal, ovarian, testicular and placental steroidogenesis or pituitary and hypothalamic hormone production (Kavlock *et al.*, 1996). The following are descriptions of a few examples of *in vitro* assays used for ranking potential teratogenicity or endocrine disruptor activity.

### Yeast Estrogen System

A simple yeast estrogen system (YES) containing human estrogen receptor (hER) is one *in vitro* technique used to screen the estrogenic potencies of chemicals or combinations of chemicals. The system consists of yeast cells engineered to contain genes that code for human estrogen receptor and a "reporter" protein that the cell makes when an estrogen-like compound binds to the receptor. The culture turns blue when a chemical binds to the receptor; the intensity of the color reflects how strongly the receptor is activated (Simons, 1996).

### E-Screen

MCF-7 human breast adenocarcinoma cancer cells have been studied extensively as a model for hormonal effects on breast cancer cell growth and specific protein synthesis. Because the proliferative effect of natural estrogen is considered the hallmark of estrogen action, it was proposed that this property be used to determine whether a substance is an estrogen mimic. The E-screen assay, developed for this purpose, uses human breast estrogen-sensitive MCF-7 cells and compares the cell yield achieved after six days of culture in medium supplemented with 5% charcoal-dextran stripped human serum in the presence (positive control) or absence (negative control) of estradiol and with diverse concentrations of xenobiotics suspected of being estrogenic (Soto *et al.*, 1995).

## Hydra Assay

The hydra assay is another *in vitro* test used to screen developmental toxicity of chemicals. This assay has demonstrated a remarkable ability to recapitulate the data of complex studies made in pregnant mammals (Johnson *et al.*, 1988). Fresh water *Hydra attenuata* are dissociated mechanically into component cells which are then randomly reassociated by low-speed centrifugation into small pellets. The pellets undergo full ontogenesis within 92 hours, resulting in a new population of adult polyps. These artificial preparations consist of two broad classes of cells: fully differentiated adult cells, which quickly achieve spatial orientation and migrate to a position consistent with their phenotype and less differentiated interstitial cells capable of becoming any of numerous differentiated cells typical of adult hydra. These interstitial cells undergo rapid and markedly localized proliferation and differentiate into tentacles and nematocysts. Within 92 hours, these artificially manufactured pellets achieve all the developmental biologic phenomena known to occur in an embryo of any species. Therefore they are termed "artificial embryos".

The first step employs test chemical concentrations at whole-log concentration intervals from 1 x $10^{-3}$ through 1 x $10^{3}$ ml/l. This step identifies the lowest whole-log concentration capable of disrupting development of the embryo and, when repeated with adults, the lowest concentration capable of producing adult toxicity. The lowest effective dose then is rerun along with a concentration one whole log lower. If the lower concentration results in no effect, the eight 1/10 log concentrations between the two levels are then run. This determines to within 1/10 log the minimal effective concentrations (MEC) of the test substance capable of producing adult (A) and developmental (D) toxicity. The adult MEC (A) and the developmental MEC (D) are then calculated as an A/D ratio. This assay provides a quick ranking order for sorting substances worthy of closer evaluation in real embryos and allows a no-observed effect level to be determined (Johnson *et al.*, 1988).

## Chick Embryotoxicity Screening Test

The chick embryotoxicity screening test (CHEST) is a fast technique requiring modest laboratory equipment, moderate skill and little expenditure of time and money. Fertilized eggs at 1.5, 2, 3 and 4 days of incubation are administered diluted test substances (using the window technique on the blunt ends) and incubated. The test procedure consists of two parts: the estimation of the embryotoxicity range (CHEST I) and the determination of the embryotoxicity parameters (CHEST II). In CHEST I, after approximately 40 hours of incubation, 3 or 10 µl of each dilution is injected subgerminally into groups of six normal embryos. After another 24 hours of incubation, the length of the newly formed part of the trunk, distance between vitelline arteries and the caudal pole of the tail are measured using a dissecting microscope. In CHEST II, three to four doses are applied to groups of ten embryos incubated for two, three and four days. The solutions are injected subgerminally on the second day and intra-amniotically on the third and fourth days. The embryos are then incubated to day 8 without turning. The following parameters of embryotoxicity are established: beginning of the embryotoxicity dose range, dose-response and stage-response relationships, proportion of dead and growth-retarded fetuses and malformation spectra in the survivors (Jelinek and Peterka, 1985).

## Current Research on EDC Screening Techniques

The International Society of Regulatory Toxicology and Pharmacology recently held a meeting entitled "Assessing the risks of adverse endocrine-mediated effects" on January 13-14, 1997 in Research Triangle Park, NC. A number of papers on potential EDC screening assays or validation of assays were presented. The following is a brief description of some of the research projects being currently conducted nationwide that were presented at that meeting.

The National Institute of Environmental Health and Science (NIEHS) has developed and is currently validating *in vitro* assays to determine the estrogenic potential of individual compounds and complex mixtures from hazardous waste sites. They have also developed and are now validating solid phase extraction, fractionation and instrumental quantification techniques to detect and quantify estrogenic and anti-estrogenic activity in complex mixtures in surface waters by use of bioassay-directed instrumental analyses (Blankenship *et al.*, 1997).

The Chemical Industry Institute of Toxicology (CIIT) has studied the effects of 17β-estradiol ($E_2$; a full estrogen receptor (ER) agonist) and bisphenol A (BPA) on the expression of estrogen-responsive genes in the endometrial carcinoma ECC-1 cell line. These studies have illustrated that BPA produces different patterns of gene expression than those observed with $E_2$. Results are expected to provide information that can be used to predict the dose-dependent actions of exogenous estrogens at the estrogen receptor (Bergeron *et al.*, 1997).

Ellington *et al.* (1997) have developed an *in vitro* co-culture system using oviduct epithelial cells (OEC) monolayers for human and animal sperm which allows seven to nine days in co-culture verses the three to four days seen in standard media. The longer survival time allows a better opportunity for detection of abnormal sperm function.

Arnold *et al.* (1996) found 150- to 1600-fold synergistic interactions between binary mixtures of the weakly estrogenic pesticides endosulfan, dieldrin, toxaphene and chlordane in competitive ER binding assays. Synergism was similarly found in an estrogen-responsive assay in yeast.

Gaido *et al.* (1997) reassessed the estrogenic activity of two of the weakly estrogenic pesticides used in the Arnold *et al.* (1996) study (i.e., a dieldrin/toxaphene mixture). Gaido *et al.* used ten different estrogen-responsive assays to assess recent reports of synergistic ER binding and activation of yeast-based reporter gene assays by binary combinations of organochlorine pesticides. The following assays were compared: induction of uterine wet weight, progesterone receptor levels and uterine peroxidase activity in the immature female mouse; induction of cell growth in MCF-7 human breast cancer cells and induction of reporter gene activity in MCF-7 cells transiently transfected of reporter gene activities in two yeast-based assays which expressed either the human or mouse ER; and competitive ER binding in MCF-7 cells and in mouse ER. For all ten assays, including the yeast-based reporter gene assay, combined activity of dieldrin and toxaphene were additive. This did not support the Arnold *et al.* YES results discussed above, which reported synergistic interactions of organochlorine pesticides.

In January 1997, the authors of Arnold *et al.* (1996) and Gaido *et al.* (1997) commented on the differences noted between their results. The differences appear to be attributed to different test methods. They suggest that synergism between weakly estrogenic chemicals is not universal, even within the same strain of yeast. The assays used by Gaido *et al.* were different from

those used by Arnold *et al.* In the Arnold *et al.* mammalian and yeast cell assays, as well as in the ligand-binding experiments, the concentration of receptor molecules was low (Ishikawa uterine cancer cells lack detectable ER and were transfected with only 20 ng of hER complimentary DNA (cDNA)), while in the study by Gaido *et al.* the concentrations were high (MCF-7 breast cancer cells with high levels of endogenous ERs). ER concentration may play an important role in the ability of mixtures of chemicals to synergize. In addition, the work performed by Arnold *et al.* showed synergism of weakly estrogenic chemicals in turtles that were treated early in development. The study by Gaido *et al.* was performed in the uteri of female mice that had already undergone sexual differentiation (Ramamoorthy *et al.*, 1997).

The 1996 Arnold *et al.* findings were formally withdrawn in July, 1997, by J. A. McLachlan, one of the co-authors. The authors had been unable to replicate their 1996 findings or find a satisfactory mechanism to explain synergy of these chemicals. Although a fundamental flaw in the design of the experiment is evident, the study generated much interest in environmental endocrinology which is continuing even after the original study has been withdrawn.

## ORGANIC SOLVENTS

### Perchloroethylene

Tetrachloroethylene or perchloroethylene (PCE) is a colorless, non-flammable, moderately volatile solvent used in dry cleaning facilities and as a degreasing agent. It is highly soluble in blood and adipose tissue and has a considerably longer half-life *in vivo* than most other solvents.

The following studies reveal reproductive and developmental effects from PCE at high exposure levels only. Statistically significant increases in morphologic abnormalities of mouse sperm have been noted with increasing exposure. The animal studies on sperm quality are supported by a number of epidemiological/occupational studies, which suggest that PCE has subtle effects on sperm quality and is associated with significant reductions in pregnancies. Whether these effects were endocrine related or due to direct DNA damage cannot be concluded from these studies. In addition, neurochemical alterations, mostly declines in acetylcholine levels, have been noted in rats exposed *in utero* at high concentrations of PCE.

#### Perchloroethylene Toxicity Studies

Studies of carcinogenicity of PCE are relevant to endocrine disruption because genotoxic carcinogens may also affect germ cells. Two positive animal studies were identified by van der Gulden and Zielhuis (1989). A study by the National Cancer Institute (NCI, 1977) resulted in a significantly increased incidence of hepatocellular carcinoma in both male and female mice after oral exposure ranging from 536 to 1072 mg/kg-day PCE for 17 months or 5 years. Similar studies with similar dosages have resulted in no affects in rats (NCI, 1977), guinea pigs, rabbits and monkeys (Rowe *et al.*, 1952). Mennear (1985), however, observed effects in rats after inhalation of 200 to 400 ppm PCE and after 100 to 200 ppm, 6 hours/day, 5 days/week for 2 years with mice. The incidence of mononuclear cell leukemia was significantly greater in rats

(both sexes) than in controls. Male rats exhibited renal tubular cell adenomas and adenocarcinomas (as cited by van der Gulden and Zielhuis, 1989).

Perchloroethylene Neuroendocrine Studies

Nelson *et al.* (1980) (as cited in Nelson, 1986) evaluated the effects of PCE on behavioral and neurochemical activity in offspring of Sprague-Dawley rats exposed to 0, 100 or 900 ppm PCE for 7 hours/day on gestation days 7 through 13 or 14 through 20. Behavioral testing was performed on days 4 through 46 and included measures of olfactory discrimination, neuromotor coordination, open field and running wheel activity, avoidance and operant conditioning. Brain samples from newborn and 21-day old offspring were analyzed for neurochemical deviations in protein, acetylcholine, dopamine, norepinephrine and 5-hydroxytryptamine. Behavioral changes were noted in higher exposure groups, primarily rotor performance and open field activity in the group exposed on days 14 through 20. Neurochemical deviations were detected after 900 ppm exposure in either exposure group, with the most pronounced changes being reductions of acetylcholine in brains of 21-day-old offspring. The changes were not significantly different from controls for the 100 ppm exposure group.

Perchloroethylene Reproductive Studies

Beliles *et al.* (1980) found that male mice, but not rats, exposed to 500 ppm of PCE had higher proportions of abnormally shaped sperm than the unexposed animals. Land *et al.* (1981) studied trichloroethylene, as well as other halogenated solvents like chloroform, and found a statistically significant increase in morphologic abnormalities in mouse spermatozoa with increasing exposure.

Perchloroethylene Developmental Studies

The National Institute for Occupational Safety and Health (NIOSH) tested 19 commonly used industrial chemicals on rats or rabbits during days 1 through 19 and 1 through 24, respectively, of gestation. PCE was one of the chemicals administered at 500 ppm for 6 to 7 hours daily. The pregnant animals were sacrificed on day 21 for rats and day 30 for rabbits. Histopathological examinations and weighing of maternal organs were conducted and uterine contents were examined for visible malformations. No evidence of maternal or fetal toxicity or teratogenicity was noted (Hardin *et al.*, 1981).

The effect of maternally inhaled trichloroethylene, PCE, methyl chloroform and methylene chloride on embryonic and fetal development in mice and rats was studied by Schwetz *et al.* (1975). Groups of pregnant rats and mice were exposed to 300 ppm trichloroethylene or PCE, 7 hours/day during days 6 through 15 of gestation. A slight but significant reduction (4 to 5%) in mean bodyweight of maternal rats was noted, but not in maternal mice, following exposure to trichloroethylene and PCE. No effect on average number of implantations sites per litter, litter size, incidence of fetal resorptions, fetal sex ratios or fetal body measurements among mice or rats were noted following exposure to 300 ppm PCE. The results suggests that these solvents were not teratogenic in either species at the administered concentrations.

Mede *et al.* (1989) examined postnatal manifestation of PCE administered prenatally. Pregnant animals were distributed into 3 groups of 15 members each; groups were exposed to different doses. The postnatal development of the offspring (4 males and 4 females per litter) was followed up to 100 days, when the animals were sacrificed. It was found that survival index was dose dependently decreased by the prenatally administered (inhaled) PCE. Autopsy on day 100 could not reveal any alteration of the offspring that could have been related to PCE exposure. It was concluded that the majority of the anomalies found on the 21st day in the offspring of rats that had been treated during the whole gestation period "disappear" postnatally due to the fact that minor anomalies are retardations or compensable variations, while major anomalies are incompatible with life.

Perchloroethylene Epidemiological Studies

Epidemiological studies support the findings of the animal studies reported above which noted significant effects on sperm morphology. Eskenazi *et al.* (1991) studied the effect of PCE on sperm quality among dry cleaning workers. They compared the semen quality of dry cleaning workers with that of laundry workers and examined the relationship of 17 semen parameters to expired air levels of PCE and to an exposure index based on job tasks in the last 3 months (i.e., the approximate period of spermatogenesis). The average sperm concentration for both groups was over eight million/ml, within normal limits based on clinical measurements; however, the sperm of dry cleaners did exhibit less amplitude of lateral head displacement (ALH) and less linearity of sperm motion than that of the laundry workers. Expired air PCE levels were positively correlated with ALH ($r = 0.41$, $p = 0.02$). The three month exposure index was positively associated with percent round sperm ($r = 0.23$, $p = 0.04$) and ALH ($r = 0.33$, $p = 0.001$). Infertility has been reported in men with mostly round headed sperm due to an inability to penetrate the ova (Syms *et al.*, 1984). PCE concentrations in expired air were not reported. This study suggests that occupational exposure to PCE could have subtle effects on sperm quality. However, the results were not highly significant. The sample population was unionized workers. Only 10-15% of U.S. dry cleaners are unionized; therefore, the results may under represent the effects of PCE exposure to dry cleaning workers.

To investigate whether exposure to trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane increases carcinogenic risk, Anttila *et al.* (1995) studied a cohort of 2050 male and 1924 female workers monitored for occupational exposure to these agents and followed up with a survey of cancer incidence during the years of 1967 to 1992. The overall cancer incidence within the cohort was similar to that of the Finnish population. There was an excess of cancers of the cervix uteri and lymphohematopoietic tissues, however. Excess of pancreatic cancer and non-Hodgkin lymphoma was seen after ten years from the first personal measurement. Among those exposed to trichloroethylene, the overall cancer incidence was increased for a follow-up period of more than 20 years. There was an excess of cancers of the stomach, liver, prostate and lymphohematopoietic tissues combined. This study does support excess cancers of the stomach, pancreas, cervix uteri, prostate and nervous system among workers exposed to solvents.

Sallmen *et al.* (1995) conducted a retrospective time-to-pregnancy study on women biologically monitored for exposure to organic solvents (trichloroethylene and PCE). The women were participants in a previous study on spontaneous abortion. They were classified in exposure categories on basis of work description, use of solvents and on biological exposure (i.e., high,

low or none). Highly exposed workers handled solvents daily or one to four days per week; exposure measurements confirmed high exposure levels. Workers with low exposure handled solvents one to four days a week but either monitoring data indicated low exposure or exposure measurements were not available for these workers. Daily or high solvent exposure, adjusted for potential confounders, was significantly associated with reduced fertility (incidence density ratio of clinical pregnancies = 0.41; 95% confidence interval (CI) = 0.27 - 0.62). The incidence density ratios were decreased also among workers who were exposed to organic solvents in shoe factories (0.28; CI = 0.11 - 0.71), dry cleaning shops (0.44; CI = 0.22 - 0.86) and the metal industry (0.58; CI = 0.34 - 0.98). The possible effects of various biases are discussed. The results of the study support the hypothesis that daily or high exposure to organic solvents is associated with reduced fertility. There is a need for safer working methods in industries where these solvents are used.

## 1,2-Dichloroethane

Most of the 1,2-dichloroethane (DCE) produced in the U.S. is used in the synthesis of vinyl chloride. It is also used in smaller amounts in the production of other chemicals, in various solvent applications, as a lead scavenger in leaded gasoline and as a fumigant for stored food products. The greatest potential for exposure to DCE in the military is from using it as a solvent for metal degreasing. Non-occupational exposure may occur from inhalation of contaminated air or by ingestion of some waters. The National Toxicology Program (NTP, 1991) observed 1,2-dichloroethane more frequently in finished water than in untreated water, suggesting that contamination may occur during water chlorination.

Few studies inferring potential endocrine disrupting activity due to DCE were found in this literature review. The studies found suggest that DCE is not a strong teratogen even at doses producing maternal toxicity. The major effects of DCE exposure appeared to be to the central nervous system and the liver.

### 1,2-Dichloroethane Toxicity Studies

The World Health Organization (WHO, 1987) reports DCE as carcinogenic in B6C3F1 mice and Osborne-Mendel rats following administration of 50 or 300 mg/kg bodyweight, given by gavage in oil. In male rats, squamous cell carcinomas of the forestomach, subcutaneous fibromas and hemangiosarcomas in several organs (mainly the spleen) were produced following gavage; in female rats, mammary gland fibromas and mammary adenocarcinomas were increased. In mice, increased incidences of hepatocellular carcinomas in males, mammary gland adenocarcinomas in females and lung adenomas in both sexes were observed. No increase in tumor incidence was reported in inhalation studies on Swiss mice and Sprague Dawley rats exposed to concentrations of up to 607 mg/m$^3$. Prolongation of the estrus cycle and increased embryo mortality, pre-implantation losses and hematomas were found when female rats were exposed to 15 mg/m$^3$, 4 hours/day, 6 days/week for 4 months prior to mating and during pregnancy. While the fetal toxicity of DCE was not confirmed at higher exposure levels, severe toxic effects on rats were observed and all implantations resorbed. No fetal abnormalities were observed in the rabbit. Oral administration to male and female rats of up to 35 mg DCE/kg bodyweight per day, via diet for up to 2 years did not affect reproduction. No effects on fertility

or gestation index and no teratological effects were observed in a two-generation study on mice treated with DCE/kg bodyweight/day via drinking water for up to 25 weeks.

### 1,2-Dichloroethane Developmental Studies

Administration of DCE either by inhalation (Rao *et al.,* 1980), by drinking water (Lane *et al.*, 1982) or in the diet (Alumot *et al.,* 1976) did not affect fertility or induce embryotoxic, fetotoxic or teratogenic effects in several species. Vosovaya (1977) observed a possible adverse effect on reproduction after exposure of female rats to DCE by inhalation at 15 $mg/m^3$, 4 hours per day, 6 days per week for 4 months before mating. During the dosing period, the length of the estrous cycle was increased. After mating the exposure was continued. An increase in the embryonic mortality and a five-fold increase in preimplantation losses were noted in the exposed rats compared to the controls. Vosovaya increased the exposure to 57 $mg/m^3$ for 4 hours per day, 6 days per week for 6 to 9 months. The fertility of mated females and the weight of newborn rats were reduced and prenatal mortality was increased.

EPA's 1985 health assessment document for DCE states that the available evidence suggests DCE does not adversely affect the reproductive or development process in laboratory animals except at maternally toxic levels. However, they suggest that additional laboratory testing is needed, as well as epidemiological studies, to conclusively establish that DCE is not a human teratogen and does not cause adverse reproductive effects. Positive responses in different test systems representing a wide range of organisms indicate that DCE is capable of causing gene mutation in prokaryotes and eukaryotes. DCE metabolites have not been adequately tested to assess ability to cause gene mutation.

## Methyl Ethyl Ketone

Methyl ethyl ketone (MEK) is a widely used industrial solvent to which there has been considerable human exposure. Few studies were found pertaining to reproductive/developmental toxicity to MEK. Signs of developmental toxicity have been noted at high levels of exposure.

Few studies were found pertaining to reproductive/developmental toxicity to MEK. Mild signs of developmental toxicity (notably delayed growth) have been noted at high levels of exposure; however, maternal toxicity was not noted at these levels. The studies do not suggest teratogenic effects at lower exposure levels. An increased incidence (although not significant) of prostate cancer was associated with MEK exposure in a cohort mortality study of male oil refinery workers.

### Methyl Ethyl Ketone Developmental Studies

Effects of subanesthetic concentrations of solvents on fetal development were studied by Schwetz *et al.* (1974). Groups of pregnant rats were exposed to carbon tetrachloride at 300 or 1000 ppm, 1,1-dichloroethane at 3800 or 6000 ppm and methyl ethyl ketone at 1000 or 3000 ppm, 7 hours/day on days 6 through 15 of gestation. Nonpregnant female rats were exposed simultaneously with the pregnant rats and were used for evaluation of treatment-related

changes in serum glutamic pyruvic transaminase (SGPT) activity during and after exposure. At both doses, the DCA exposure had no effect on the incidence of fetal resorptions, fetal body measurements or on the incidence of gross or soft tissue anomalies. There was a significantly increased incidence of delayed ossification of sternebrae associated with exposure to 6000 ppm DCA. There was a slight but significant decrease in food consumption and weight gain in both dose groups; but no effects on conception rate, number of implantations or litter size, SGPT activity or appearance of the liver were found. At both exposure levels, MEK did not affect the incidence of fetal resorptions. Exposure to 3000 ppm MEK caused a low incidence of true malformations (e.g., acaudia (missing tail), imperforate (closed) anus, retarded fetal development and brachygnathia). A significant increase in incidence of anomalies indicative of retarded fetal development was observed among litters of dams exposed to MEK; however, no measurable maternal toxicity was noted. This suggests that there was no correlation between the toxicity incurred by the mother and that incurred by the fetus with these solvents since MEK, which was least maternally toxic, demonstrated a potential to cause terata.

Schwetz *et al.* (1991) conducted a similar experiment using lower concentrations of MEK. Swiss mice were exposed to 0, 400, 1000 or 3000 ppm MEK vapors 7 hours/day on days 6 to 15 of gestation. Groups consisted of about 30 pregnant females each. Overt maternal toxicity at any concentration was not noted; however, there was a significant increase in liver weight in the 3000 ppm group. Developmental toxicity (as reduced fetal bodyweight of males) was also significant in the 3000 ppm group. No increase in resorption rate was noted. Several malformations were observed at very low incidence (e.g., cleft palate, fused ribs, missing vertebrae and syndactyly); however the incidence was significant only for misaligned sternebrae at 3000 ppm.

A neurotoxicity study by Stoltenburg-Didinger *et al.* (1990) established a dose dependent increase in embryotoxicity and fetotoxicity from MEK and n-hexane exposure. Rats were exposed to MEK (800 or 1000 to 1500 ppm) and/or n-hexane either prenatally, postnatally or during gestation. A serious increase in intrauterine mortality was observed as less than two-thirds of the rats exposed to MEK brought forth young as compared to 100% of the controls. Both the pregnancy rate and resorption rate showed marked relationships to the exposure concentration. In the prenatal exposure cases, reduced body growth was observed at all concentrations investigated (500 ppm, 800 ppm and 1000 - 1500 ppm, 23 hours/day for 21 prenatal days). A delay in the maturation of cerebellar cortex was also observed. In cases of pre- and postnatal exposure to a mixture of MEK and n-hexane, bodyweight became significantly lower with time as compared to that of the controls. This resulted in delayed tissue maturation. No teratogenic effect in pregnant rodents and their offspring were noted from the solvents.

Mast *et al.* (1989) exposed four groups of Swiss CD-1 mice to 0, 400, 1000 or 3000 ppm MEK for 7 hours/day. Ten virgin females and approximately 30 pregnant females per group were exposed concurrently for 10 consecutive days (gestation days 6 through 15 for mated mice). On day 18 of gestation, mice were sacrificed and maternal bodyweight, uterine weight and fetal bodyweights were obtained. Uterine implants were enumerated and their status recorded. Live fetuses were sexed and examined for defects. The doses did not result in apparent maternal toxicity although there was a slight, treatment correlated increase in liver to bodyweight ratios which was significant for the 3000 ppm group. Mild developmental toxicity was evident at 3000 ppm as a reduction in mean fetal bodyweight. This reduction was statistically significant for the males only. Although there was not an increase incidence of intrauterine death in the fetuses of

mice exposed to MEK, there was an increased incidence of misaligned sternebrae correlated to increasing exposure concentration (statistically significant for 3000 ppm). Several malformations (e.g., cleft palate, fused ribs, missing vertebrae and syndactyly) were noted at low incidence but were not statistically significant. Offspring exhibited significant signs of toxicity at 3000 ppm.

### Methyl Ethyl Ketone Epidemiological Studies

A retrospective cohort mortality study of male oil refinery workers who had worked on lubricating-dewaxing processes was performed by Wen *et al.* (1985). The study consisted of 1,008 individuals with 21,795 person-years of observation and 43 years (1935-1978) of follow-up. The workers were exposed to several solvents, primarily MEK and toluene, at levels below Occupational Safety and Health Administration (OSHA) standards. The standardized mortality ratio (SMR) for all causes and for cancer were much lower than unity when compared to U.S. population mortality rates. However, eight prostate cancer deaths were noted (4.4 expected), yielding a SMR of 1.82. Only one of these eight worked on a MEK unit. The remaining seven had lube oil department-wide assignments. This study presented a favorable mortality pattern among oil refinery workers; however it did identify an increased incidence of prostate cancer which could possible be related to MEK solvents, lubricating oil or other products.

## Methyl Isobutyl Ketone

Methyl isobutyl ketone (MIBK) is a volatile, flammable liquid used as an intermediate in organic synthesis and as a solvent in industrial products and for extraction. Only one study pertaining to developmental toxicity of MIBK was found; this study indicated slight behavioral alterations of offspring exposed *in utero.* No data concerning effect on reproduction or chronic toxicity have been found. Thus long term effects need to be investigated.

### Methyl Isobutyl Ketone Developmental Studies

Peters *et al.* (1981) exposed groups of 25 pregnant Fischer 344 rats by inhalation to 0, 500, 1000 or 2000 ppm MIBK for 6 hours/day throughout gestation. Typically, five pups per treatment group were tested for simple reflexes, open field and running wheel activity, food maze behavior (not defined), swimming stress and avoidance conditioning. Some were also tested for pentobarbital-induced sleeping time, clinical chemistry and histopathology. The group exposed to 500 ppm MIBK was discarded for a non-treatment related nutritional problem. The two higher concentrations produced decrements in maternal weight gain and in the number and weight of offspring. Behavioral alterations were detected in most, with offspring from exposed animals having reduced activity in the open field, increased activity in the running wheel and deficits in avoidance conditioning. A few sporadic changes were also noted in clinical chemistry values and histopathology, but few consistent dose-related effects were apparent.

## Trichloroethylene

Trichloroethylene (TCE), a volatile organic compound commonly used as an industrial degreaser and in research laboratories, is among the most common water supply contaminants in the U.S. and abroad. Over the past 15 years the carcinogenic potential of TCE has been studied extensively; however, the literature on reproductive effects, especially by oral administration, is limited.

Several reproductive/developmental studies on TCE were found in this literature search. TCE appears to be a cardiac teratogen when exposure occurs during the period of organ differentiation and development. Epidemiological studies have also associated halogenated hydrocarbons with increased incidence of cardiac deformities. Fertility did not appear to be affected even from high exposures (1000 mg/kg-day).

### Trichloroethylene Toxicity Studies

Kjellstrand *et al.* (1985) studied the effect of TCE on butyrylcholinesterase (BuChE) activity and the effects of testosterone and sex hormone binding globulin (SHBG) on animals exposed to TCE. In one experiment, NMRI mice were exposed to either 75 ppm PCE, 625 ppm 1,1,1-trichloroethane or 15 ppm trichloroethylene. In a second experiment, mice (castrated 7 days prior) were delivered 0.5 µl/hour testosterone (concentration of 18 mg/ml) for 14 days via osmotic minimums placed subcutaneously on the abdomen. Increases in activity of plasma BuChE are seen in males but not females at low concentrations of TCE. The significant difference seen after PCE exposure in this experiment is probably due to an unusually low BuChE in the corresponding control group. Liver weight increase was also commonly seen in the groups (both males and females) exposed to TCE. There was no correlation between the appearance of the livers and weight increase. However, after exposure to PCE, the livers were yellowish, while they looked normal but enlarged after TCE exposure. Kidney weights were much lower after exposure to TCE. In castrated mice, the liver weight was 1.18 times higher in the testosterone-treated castrates than in the non-treated castrates ($p<0.001$). The difference was approximately the same as that between the castrated group exposed to TCE and the corresponding group that also was given testosterone (i.e., liver weight 1.25 times higher among castrates) ($p<0.001$). Liver and kidney weights were considerably lower in the castrated group than in the corresponding control ($p<0.001$).

Increases in the activity of plasma BuChE were seen in male but not in female mice on exposure to low concentrations of TCE. This effect seems to be the most sensitive response to exposure thus far observed and is even more sensitive than liver weight increase. The dose response curve is linear up to rather high exposure concentrations. Depletion of testosterone through castration or destruction of the pituitary gland or hypothalamus are the only other ways to experimentally induce corresponding increases in BuChE. Plasma BuChE activity increase was found to be a common reaction after exposure to TCE or PCE. 1,1,1-Trichloroethane had little or no effect on BuChE activity. Normal and castrated male mice were continuously exposed for 4 weeks to 150 ppm TCE. The increase in BuChE activity in castrated males was not further increased by TCE exposure. Administration of testosterone with osmotic minipumps for 13 days nearly restored the normal testosterone and BuChE levels in castrates. The effect of TCE exposure on BuChE activity in these animals was the same as on normal males. Testosterone levels were not influenced by the TCE exposure in normal males or in castrated

males given testosterone. Unlike rabbits, no SHBG could be detected in the mice. There seems to be no correlation between the presence or absence of SHBG and effects on the activity of BuChE in different species. Although BuChE activity appears to react after experimental manipulation of the testosterone levels in the same way as SHBG, changes induced through solvent exposure are neither directly nor indirectly (through SHBG) due to effects on testosterone. The results from these animal experiments do not support the epidemiological findings of decreased testosterone levels in humans exposed to solvents. On the contrary, increases in BuChE activity have been shown to be independent of testosterone activity (Kjellstrand *et al.*, 1985).

Trichloroethylene Reproductive Studies

The reproductive toxicity of micro-encapsulated TCE (0, 0.15, 0.3 or 0.6% in feed) was evaluated in CD-mice and F344 rats by the continuous breeding protocol. Mice ingested twice the amount of TCE (mg/kg-day) as rats. After 18 weeks of exposure, mice had normal fertility; live pup weight was decreased at 0.6% TCE. At necropsy, 0.6% TCE mice had increased relative liver weight, decreased sperm motility, TCE-related hepatic hypertrophy and renal tubular degeneration. The first generation 0.6% TCE mice had normal fertility rates but showed a significant preweaning loss of entire litters. At 0.6% TCE, relative weights of liver, kidney and testis were increased, sperm motility was decreased and hepatic and renal lesions similar to adult mice were observed. In adult rats, TCE caused a dose-related trend for decreased live litters/pair and live pups/litter. First generation rats exposed to 0.15 through 0.6% TCE had normal fertility. At necropsy, bodyweight was decreased and relative liver weight was increased in all TCE-treated groups. No significant pathology was noted. At the maximally-tolerated doses, TCE had minimal reproductive toxicity in both mice and rats (George *et al.*, 1990).

Trichloroethylene Developmental Studies

The effect of subchronic oral exposure to TCE on female reproductive performance and whether TCE metabolites (trichloroacetic acid (TCA) and trichloroethanol (TCOH) preferentially accumulate in female reproductive organs or neonatal tissues was studied by Manson *et al.* in 1984. Female Long-Evans rats were exposed to 10, 100 or 1000 mg/kg per day TCE in corn oil by gavage for 2 weeks before mating and through gestation day 21. Gas chromatograph (GC) analysis of tissues from females at the end of premating exposure indicated that TCE levels were uniformly high in fat, adrenal and ovarian tissue in each treatment group, while uterine tissue had relatively high levels of TCA. Female fertility, however, was not affected in any treatment group. In the 1000 mg/kg-day group, 5 of 23 females died and weight gain was significantly depressed throughout the treatment. Neonatal survival was significantly depressed in this group alone, with the majority of deaths occurring among female offspring at the time of birth. There was an increase in TCA levels in blood, liver and stomach contents with increasing treatment levels in the female, but not male, neonates. The results indicate that oral exposure to TCE below the dose causing maternal toxicity had no influence on pregnancy outcome and that the accumulation of TCE and TCA in ovaries, adrenals and uteri had no influence on mating success.

Hardin *et al.* (1981) exposed rats and rabbits on gestation days 1 through 19 and 1 through 24, respectively, to 500 ppm trichloroethylene for 6 or 7 hours daily. The pregnant animals were

sacrificed on day 21 for rats and day 30 for rabbits. Histopathological examinations and weighing were conducted on maternal organs and uterine contents were examined for visible malformations. There was no significant change in the malformation rates in either species, but four cases of external hypocephaly were noted among the trichloroethylene-exposed rabbits. External hypocephaly in rabbits is rare and raises suspicion of a teratogenic response. TCE had previously been tested at a lower concentration of 300 ppm. The results of that study were negative for both species.

Trichloroethylene appears to be a cardiac teratogen when exposure occurs during the period of organ differentiation and development. Epidemiological studies have found an association between halogenated hydrocarbons and increased incidence of major cardiac malformations in children born to mothers living in areas of water contamination. Dawson *et al.* (1990) examined the effect of trichloroethylene and dichloroethylene on cardiac development by delivering TCE and DCE in saline solutions through a catheter to the gravid uterus during organ differentiation. Rats were divided into 5 dose groups: 1500 ppm TCE, 15 ppm TCE, 150 ppm DCE, 1.5 ppm DCE and a saline control group. The same quantity (200 μl) of solution was administered at a rate of 0.5 l/hour over a 2 week period in each dose group. Litter size showed no correlation with treatment. Cardiac effects were dose dependent. A very small number of congenital heart anomalies (3%) were found in the control group; 9% and 12.5% were found in the lower dose trichloroethylene and dichloroethylene groups and 14% and 21% in the higher dose groups, respectively ($p<0.05$). Several types of cardiac defects were noted; however, no noncardiac anomalies were detected, suggesting that these agents are specific cardiac teratogens.

Healy and Wilcox (1978) exposed pregnant rats to TCE in a concentration of 100 ppm in air during a period of 4 hours/day, 7 days/week from day 6 through day 20 of pregnancy. An association between inhalation of TCE and reduced fetal weight at 20 days and also an increase in the number of fetuses resorbed were observed ($0.001<p<0.01$).

In a second paper, Healy *et al.* (1982) exposed 32 Wistar rats to 100 ppm trichloroethylene for 4 hours/day from days 8 to 21 of pregnancy. A control group of 31 pregnant rats was exposed to the same conditions without the TCE. All rats were sacrificed on day 21. There was not a significant difference in the frequency of fetal loss or in litter size. However, the number of rats in which total resorption of all fetuses occurred was greater in the exposed group ($p<0.05$). Fetal weight was reduced ($p<0.05$) but crown-rump length was not ($p<0.1$), suggesting that TCE may retard fetal development. The difference in the ratio of male to female fetuses was not significant. No external visceral or gross skeletal anomalies were found in either group.

Another study of the effect of maternally inhaled TCE, PCE, methyl chloroform and methylene chloride on embryo and fetal development in mice and rats was conducted by Schwetz *et al.* (1975). Groups of pregnant rats and mice were exposed to 300 ppm TCE or 300 ppm PCE, 7 hours/day during days 6 to 15 of gestation. A slight but significant reduction (4 to 5%) in mean bodyweight of maternal rats was noted in rats, but not mice, following exposure to TCE and PCE. No effect on average number of implantations sites per litter, litter size, incidence of fetal resorptions, fetal sex ratios or fetal body measurements among mice or rats were noted following exposure to 300 ppm TCE. These parameters were not altered except for a significant decrease in fetal bodyweight of mice and a slight but statistically significant increase in the incidence of resorption. The results suggest that these solvents were not teratogenic in either species at the administered concentrations.

Dorfmueller *et al.* (1979) studied whether long-term exposure to TCE before mating and during pregnancy caused more adverse reproductive effects than exposure during pregnancy alone. Four treatment groups of female Long-Evans rats were exposed to TCE vapors at 1800 ±200 ppm per the following regimens: pre-mating TCE exposure for 6 hours/day, 5 days/week for 2 weeks until pregnancy was confirmed and then continued on a daily basis during the first 20 days of pregnancy; premating TCE exposure and filtered air during pregnancy; exposure to filtered air before mating and TCE during pregnancy; and filtered air before mating and during pregnancy. Incomplete ossification of the sternum was significantly increased in the group exposed during pregnancy alone, indicating delayed development rather than a true malformation. Behavior evaluation of offspring of all groups indicated a lack of treatment effect in tests of general activity levels at 10, 20 and 100 days of age. However, a reduction in postnatal bodyweight was seen in offspring from the premating exposure group. Blood analysis performed on maternal rats at sacrifice did not indicate any significant treatment effects on kidney or liver function. No results revealed maternal toxicity, embryotoxicity, severe teratogenicity or significant behavioral deficits. Analysis of mixed function oxidase (MFO) enzymes in fetal and maternal tissues revealed a greater induction of enzyme activities in long-term exposure compared to short-term exposure either before mating or during pregnancy alone. This enzyme activity was compared in the pregnant and non-pregnant rat livers to see if metabolism of TCE was altered during pregnancy. Elevation of ethoxycoumarin dealkylase activity in TCE-treated non-pregnant rat livers was noted but not in pregnant rat livers. This was expected because pregnant rats are resistant to induction of cytochrome P-450 activity, perhaps due to high levels of endogenous steroid hormones which compete with xenobiotics for metabolism. Ethoxyresorufin dealkylase induction was present in pregnant but absent in non-pregnant rat livers.

Reproductive effects of TCE, administered by oral gavage to pregnant mice, were studied by Cosby and Dukelow (1992). Doses of 1/10 and 1/100 of the $LD_{50}$ were administered on days 1 through 5, 6 through 10 or 11 through 15 of gestation. Organ and bodyweights of mice were recorded. Litters were counted, sexed, weighed and measured for crown-rump length until weaning on day 21. Some animals (two from each dose group) were allowed to develop to six weeks of age. Differences in gonads were recorded. No maternal or reproductive effect of TCE was seen at any of these dose levels.

Trichloroethylene *In Vitro*/Screening Studies

Bross *et al.* (1983) studied the effects of low dosages of TCE (1 through 25 µmol/egg) on chick development when embryos were exposed on days 1 and 2 of incubation and examined at day 14 of embryogenesis. The low doses of TCE (i.e., 1, 5, 10 or 25 µmol/egg) produced 50% mortality, unlike Elovaara *et al.* (1979, described in the 1,1,1-trichloroethane section) who reported an $LD_{50}$ for chick eggs to be between 50 and 100 µmol. The differences in administration of TCE apparently account for this discrepancy. Survivors at all doses exhibited these developmental defects in varying degrees which were significantly different from control embryos: evisceration, subcutaneous edema, light pigmentation of the epidermis, beak malformations, club foot and patchy feathering. The results demonstrate both potential embryotoxicity and teratogenicity of TCE in chick embryos at very low doses.

In a second set of studies, Cosby and Dukelow (1992) added TCE and it metabolites, dichloroacetic acid (DCA), TCA and TCOH, to culture media containing oocytes and

capacitated sperm to assess the toxic effects on *in vitro* fertilization in mice. Doses for each chemical were 100 ppm and 1000 ppm. The number of oocytes fertilized in both the low and high dose TCE cultures were lower, but not significantly different, from the control. TCA caused significantly less fertilization than the control at the high dose ($p<0.001$). DCA resulted in significantly lower fertilization rates for both the low ($p<0.025$) and high-dose groups ($p<0.010$). When TCOH and TCA were combined, the effect was not synergistic. The results of this study demonstrate that the effects noted *in vitro* may not even be detected *in vivo*; fertilization effects by metabolites were detected *in vitro*, but no reproductive or teratogenic effects were detected *in vivo*.

## 1,1,1-Trichloroethane

1,1,1-Trichloroethane (TRI) is a volatile chlorinated hydrocarbon. It has been increasingly used as an industrial solvent and in consumer products such as spot removers. It is estimated that approximately 88% of annual production in the U.S. is released largely to the atmosphere through dispersive use. It is less frequently detected in water. It is not soluble to any appreciable extent.

Developmental studies on TRI showed no conclusive evidence of endocrine activity. No major changes in incidence of fetal malformations or material toxicity were noted in rabbits or rats exposed during gestation to TRI. No evidence of sperm abnormalities was noted in rodents. Screening by TRI injection into incubating chick eggs, however, resulted in a number of malformations.

### 1,1,1-Trichloroethane Reproductive Studies

Topham (1980) tested 54 compounds from a wide range of chemical classes for induction of sperm-head abnormalities in mice. TRI was included in the study. No evidence of sperm abnormalities were identified at injected doses of 0.1, 0.25, 0.5, 1.0 or 1.5 mg/kg-day of TRI.

### 1,1,1-Trichloroethane Developmental Studies

Pre- and postnatal developmental effects in Sprague-Dawley rats were studied by George *et al.* (1989) in an effort to assess the repeatability of a report by Dapson *et al.* (1984) that indicated 10 ppm TRI in water caused cardiac malformations in offspring. The rats were exposed to 3, 10 and 30 ppm TRI in drinking water with 3% 1,4-dioxane as an emulsifying agent. Males and females (30 per group) were exposed to the control solutions or test compound for 14 days prior to cohabitation and for 13 days during the cohabitation phase. Pregnant females (24 to 30 per group) continued to be exposed through gestation day 21. Parent rats exhibited a slight aversion for the 30 ppm water. No significant effects on reproduction indices or postnatal growth and development of 21-day-old offspring were noted. A slight increase in mortality from implantation to postnatal day 1 was noted in the 30 ppm group. No indication of an increased incidence of cardiac malformations in postnatal day 21 pups was found.

Another study evaluating the effect on male or female reproductive function and fetal development after subchronic administration of TRI in drinking water was conducted by Lane *et*

*al.* (1982). Male and female ICR Swiss mice were given drinking water with either DCE (0, 0,03, 0.09 or 0.29 mg/ml) or TRI (1, 0.58, 1.75 or 5.83 mg/ml). The mice were allowed food and TRI solution freely; however, twice-weekly fluid consumption data were collected. For TRI, these concentrations represented daily doses of 0, 100, 300 or 1000 mg/kg. No taste aversions were noted. There appeared to be no dose-dependent effects on fertility, gestation, viability or lactation indices. Pup survival and weight gain on postnatal day 21 were not adversely affected. Gross necropsy of male and female first generation mice treated with DCE or TRI failed to reveal compound or non-related effects.

Maurissen *et al.* (1994) evaluated cognitive and other neurobehavioral effects of maternally administered 1,1,1-trichloroethane on rat offspring. Dams were gavaged with 0, 75, 250 and 750 mg/kg per day of TRI from gestation day 6 through lactation day 10. Twenty litters per dose were used and were culled to eight pups on postnatal day 4. Pups were weighed and examined for physical maturational landmarks. Motor activity, auditory brainstem response, functional observational batter, brain measurements (weight, length and width) and neuropathology were assessed on several occasions. Finally, learning, test performance and short-term memory (delayed matching-to-position paradigm) were tested at two and three months of age. Statistically significant decreases in pup weights were noted, but were not considered biologically significant. There were no effects attributable to treatment on physical development, motor activity, auditory brainstem response, neuropathology and brain measurements. 1,1,1-Trichloroethane did not affect short-term memory, learning or performance in any of the treatment groups.

1,1,1-Trichloroethane *In Vitro*/Screening Studies

Giliani and Diaz (1986) studied the effects of TRI on the development of chick embryos. TRI was dissolved in olive oil and injected into fertile chick eggs at doses of 5, 25, 50 and 100 µmol per egg. The injections were made into the air sacs of eggs on days 0 and 1 of incubation. Control eggs were injected with an equivalent volume of olive oil (0.1 ml per egg). Embryos were examined at day 13. The percent survival of the treated embryos ranged from 52 to 19% in day 0 eggs and 59 to 42% in day 1 eggs. The following malformations were observed: everted viscera, anophthalmia, exencephaly, microphthalmia and reduced body size. Teratogenicity of TRI was highest in the embryos treated on day 0.

Developmental toxicity of inhaled 1,1,1-trichloroethane in New Zealand white rabbits was evaluated by EPA (Bushy Run Research Center, 1987). Groups of 22 mated female rabbits were exposed, 6 hours/day on gestation days 6 through 18 at concentrations of 0, 1017, 3122 or 5906 ppm TRI. Mortality was not observed in any group. The clinical signs of maternal toxicity were loose feces (2 does at 5906 ppm), reduced weight gain and reduced total weight gain corrected for gravid uterus (3122 and 5906 ppm groups). No treatment induced abortions or early deliveries resulted in any group. Total resorptions in the 4 groups were 0, 5, 0 and 1, respectively. No treatment-related effects were found in does at necropsy on gestation day 29. There were no significant changes in corpora lutea counts; total, nonviable, or viable implants/litter; sex ratio; pre- or postimplantation loss or fetal bodyweights/litter. There were no significant changes in incidence of malformations individually, by category or in total. There were no treatment-related changes in incidence of fetal variations, except for increased bilateral incidence of extra bilateral 13th ribs at the high dose.

## DEICING/ANTI-ICING AGENTS

The U.S. Air Force currently has the option of several aircraft deicing/anti-icing systems, including chemical deicers, air blasting from deicing trucks or radiant heat application, to name a few. Similarly, different runway deicing methods are available; chemical deicers including glycol mixtures, mechanical deicers (i.e., snow plows) and runway ice detection systems (RIDS) are all options. In this report, ethylene and propylene glycol were selected for review as aircraft deicers/anti-icers. Also reviewed were potassium acetate, urea, calcium magnesium acetate and sodium acetate or formate, all of which are runway deicing options open to Air Force bases (Baca and Herring, 1996).

### Ethylene Glycol

Ethylene and propylene glycol are the basis of aircraft deicing chemical agents used in North America. Additionally, either glycol may be used as a runway deicer or as a pre-wetting agent for solid deicers. Both are used in automotive antifreeze. Ethylene glycol is effective at low temperatures and can melt through existing snow and ice. Ethylene glycol has a relatively high biochemical oxygen demand (BOD); propylene glycol's BOD is even higher. Ethylene glycol is potentially toxic to both mammals and aquatic life. Concentrations in runoff are typically low enough not to cause direct toxicity to aquatic life (Mericas and Wagoner, 1996). However, concern over ethylene glycol toxicity to humans, animals and pets has caused promotion of propylene glycol use. U.S. Air Force policy requires phasing out ethylene glycol-based fluids in favor of propylene glycol, due to the potential of human toxicity resulting from airfield runoff (McKenna *et al.*, 1996).

In a review of several articles on ethylene glycol toxicity, many of which are included here, Carney (1994) proposed a model for developmental toxicity based on the information reviewed. Briefly, Carney noted that developmental toxicity is preceded by an accumulation of glycolic acid, a principle metabolite of ethylene glycol. Absorption through dermal and inhalation routes is relatively poor, resulting in low peak glycolic acid blood levels. Oral absorption is more complete and oral gavage results in very high peak blood levels. Glycolic acid accumulates as its conversion to glyoxylic acid is ethylene glycol metabolism's rate limiting step. Metabolic acidosis results (Carney, 1994). Correction of acidosis with sodium bicarbonate alleviates developmental results to some degree (Khera, 1991). Carney (1994) was uncertain as to whether acidosis was the cause of all developmental toxicity; hyperosmolality may be a consideration and ethylene glycol may be directly fetotoxic. Lamb *et al.* (1985) speculated on the bone development effects of oxalic acid, another metabolite.

Ethylene glycol clearly causes embryotoxicity and teratogenicity in both *in vivo* and *in vitro* studies, although the effects usually result from relatively high dose levels. Ongoing investigations of ethylene glycol mechanisms, as discussed above, do not suggest hormonal activity. Although a developmental toxicant, no evidence of direct endocrine involvement has been found.

Ethylene Glycol Developmental Studies

Ethylene glycol was found to cause delayed skeletal ossification and increased skeletal malformations which did not persist beyond 21 days after birth. CD (Sprague-Dawley) rats were gavaged with 2500 mg ethylene glycol/kg bodyweight daily on gestation days 6 through 15. Sacrifices were made on days 18 and 20 of gestation and on postnatal days 1, 4, 14, 21 and 63. Exposed dams had decreased weight gain throughout treatment and gestation; gestation length was not effected. Offspring had significantly decreased bodyweights on gestation days 18 and 20 and on postnatal day 1, as compared to distilled water exposed controls. Delayed ossification, particularly of the vertebral centra and the sternebrae, was observed from late gestation through 21 days of age. The delay in ossification on gestation days 18 and 20 were found to be related to decreased bodyweight; however the postnatal effects seen up to day 21 were due directly to ethylene glycol exposure. Ossification abnormalities were dramatically reduced on postnatal day 63, as cartilage growth is still possible up to this age. Rib malformations were seen from gestation day 18 through postnatal day 21; malformations included short 13th ribs, fused ribs and missing ribs. Skeletal remodeling, seen with other chemical exposures, may be responsible for the absence of malformations at 63 days of age. Compensatory growth and development (i.e., catching up) can occur after birth; abnormalities normally listed as malformations may not be permanent defects (Marr *et al.*, 1992).

Metabolic acidosis was found to contribute to abnormalities caused by ethylene glycol. Cannulated Sprague-Dawley rats were orally exposed to 1250, 2500 or 5000 mg ethylene glycol/kg bodyweight with or without sodium bicarbonate on day 11 of gestation. Bicarbonate (530 mg/kg) was administered via gavage, followed by 2.65 mg sodium bicarbonate/ml drinking water for 9 hours post-exposure. All dose levels caused hyperosmolarity and metabolic acidosis within one hour of dosing. Administration of bicarbonate significantly decreased but did not eliminate these effects. At 5000 mg ethylene glycol, depressed reflexes, lack of coordination and lethargy were especially noticeable; all dams exposed to this high dose died, except those treated with sodium bicarbonate. Additional rats were gavaged with 500 mg ethylene glycol/kg daily on gestation days 7 through 13. No bicarbonate was provided. Fetal weight was significantly decreased. A high incidence of rib and vertebral effects were noted. Also reported was a low, but significant, incidence of cleft lip and palate, absent eyes and skull deformities. Abnormalities were also seen in the extraembryonic tissue (i.e., tissues surrounding the fetus, including the placenta, that are necessary for fetal survival) (Khera, 1991).

In the same study, subcutaneous exposures were also assessed. Cannulated rats were injected with 3333 mg ethylene glycol/kg bodyweight on day 11 of gestation. Sodium bicarbonate was administered to half the exposure group via gavage and drinking water in the manner described above. Similar results, including lack of coordination, decreased reflexes and lethargy, were obtained as with oral exposure to ethylene glycol. Metabolic acidosis was again achieved within an hour; bicarbonate significantly ameliorated ethylene glycol effects. Additionally, extraembryonic tissue was reported to be decreased in size as compared to distilled water injected controls. A separate group of rats were exposed to 2800 or 3333 mg ethylene glycol/kg on day 11. Sodium bicarbonate was administered in the same fashion as before to half the exposure group except administration in drinking water was continued for 24 hours. Fetal effects indicative of delayed development including decreased bodyweight, increased rib malformations (i.e., decreased numbers of ribs or fused ribs) and retarded

ossification (e.g., sternebrae, vertebrae and phalanges) occurred at both dose levels in a dose dependent manner. Additionally, at the high dose, some maternal death was observed. Administration of sodium bicarbonate again significantly decreased ethylene glycol effects. Sodium bicarbonate is an endogenous compound that helps to correct metabolic acidosis in humans (Khera, 1991).

Postnatal development was assessed in rats exposed to ethylene glycol *in utero*. Dams were exposed to 250, 1250 or 2250 mg ethylene glycol/kg bodyweight daily on gestation days 6 through 20. Pups were fostered to unexposed dams at one day of age. Fetal or maternal toxicity was not observed at the low dose. Maternal toxicity, including decreased weight, renal toxicity and increased gestation length, was seen in the mid and high dose groups. Developmental toxicity was not present at 1250 mg/kg per day. Fetal deaths were increased and pup weights were decreased at the high dose. Litter size, postnatal pup weight and viability were decreased among high dose pups up to four days of age. Axial skeletal malformations, determined on postnatal day 22, were also increased within this group. Ethylene glycol exposure had no effect on pup performance during wire grasp, exploratory or visual discrimination tasks (Bates *et al.*, 1990).

In an oral bolus study reviewed by Carney (1994), rats were dosed daily with 253, 638, 858, 1078 or 1595 mg ethylene glycol/kg bodyweight. Dosing was performed on days 6 through 15 of gestation. Maternal effects were noted at 1595 mg/kg per day. Fetal effects occurred at 858 mg/kg per day; effects included decreased bodyweights and sternum abnormalities. Placental weights were decreased at 1078 or 1595 mg/kg per day, which indicated placental involvement in developmental toxicity. The maternal and fetal no observable effect levels (NOELs) were 1078 and 638 mg/kg per day, respectively (Yin *et al.*, 1986).

In the same article Carney (1994) reviewed a feeding study by Maronpot *et al.*, 1983. Fischer 344 rats were exposed to target dose levels of 40, 200 or 1000 mg/kg-day in the diet. Dams were fed this diet on days 6 through 15 of gestation. Maternal toxicity or embryolethality was not found. Skeletal variations, including poorly ossified and unossified vertebral centra, were significantly increased in the 1000 mg/kg-day dose group.

Ethylene glycol was not found to have any reproductive effect in a three generation study by DePass *et al.*, 1986. Fischer 344 rats ($F_0$) were exposed to 0.04, 0.2 or 1.0 g ethylene glycol/kg bodyweight daily in the diet beginning approximately 50 days prior to mating at 100 days of age. Concentrations of ethylene glycol in the diet were altered according to natural food consumption changes so that intake rate remained the same. At 21 days of age, $F_1$ was weaned; this generation was co-mated at 100 days of age. $F_2$ was treated in the same manner as the two previous generations. Ethylene glycol did not effect bodyweight gain or food intake in any of the treated generations as compared to control generations. No mortality was seen in parental rats. Percent fertility and pup survival were not affected by ethylene glycol exposure either. Pup bodyweights were monitored on postnatal days 4, 14 and 21; this parameter was also not affected. No treatment related lesions were seen in $F_2$ parents or $F_3$ weanlings; kidney lesions, a reported outcome of ethylene glycol exposure, were not increased in frequency or severity as compared to controls. Additionally, a dominant lethal mutagenesis assay was completed using $F_2$ males. $F_2$ males at 155 days of age were mated with untreated females. As a positive control, untreated (control) $F_2$ males were injected intraperitoneally with 0.50 g triethylenemelamine prior to mating with untreated females. Dams were sacrificed on day 12 of gestation. No statistically significant adverse effects were seen among fetuses sired by

ethylene glycol treated $F_2$ males. Dominant lethal assay parameters included the number of females with implants, total implants, dead implants and live implants. The positive control group was severely affected in nearly all of these categories. The authors noted that ethylene glycol administered in the diet could have a different rate of absorption than ethylene glycol administered in other vehicles.

An oral developmental NOEL for ethylene glycol in rats was established at 500 mg/kg-day by Neeper-Bradley *et al.* (1995). CD dams were gavaged daily with 150, 500, 1000 or 2500 mg ethylene glycol/kg bodyweight on days 6 through 15 of gestation. Sacrifice occurred on day 21. Maternal bodyweights were significantly reduced throughout treatment and the remainder of gestation in the high dose group. Water consumption was significantly increased in this group also. Gravid uterine weight was decreased while absolute kidney weight, relative kidney weight and relative liver weight were all increased among the high dose dams. Only relative liver weight was significantly increased among rats in the 1000 mg/kg-day dosing group. No maternal treatment related lesions were seen at any dose level. Fetal bodyweights were significantly decreased in both the 1000 and 2500 mg/kg-day exposure groups. Significant increases of soft tissue abnormalities were observed only among the high dose group; abnormalities included hydrocephaly, abdominal muscular defects and umbilical hernias. Skeletal malformations and ossification abnormalities occurred in both the 1000 and 2500 mg/kg-day groups in a dose dependent manner; the majority of either disorder occurred in the thoracic region, especially involving extra or missing ribs and fused or missing thoracic arches.

In the same study, an oral developmental NOEL was established for mice at 150 mg/kg per day. CD-1 dams were orally administered 50, 150, 500 or 1500 mg ethylene glycol/kg bodyweight daily on gestation days 6 through 15. Mouse sacrifice was on day 18 of gestation. Maternal bodyweight gain, water consumption and gravid uterus, kidney or liver weights were not affected by treatment. Fetuses in the high dose group had decreased bodyweights. Soft tissue abnormalities were not increased in any treatment group. Multiple skeletal malformations and ossification abnormalities were present in the high dose group, again mainly occurring in the thoracic region. The bilateral occurrence of extra 14th ribs was highly significant in the 500 mg/kg-day dose group; no other malformations were significant in this treatment group (Neeper-Bradley *et al.*, 1995).

Higher doses and dose dependent increases in effects were seen in a study by Price *et al.* (1985). CD rats were exposed daily via gavage to 1250, 2500 or 5000 mg/kg on gestation days 6 through 15. Sacrifices were made on day 20. Overt maternal toxicity was not found. However, maternal weight gain throughout treatment was significantly decreased at all dose levels. Water consumption was significantly increased throughout the duration of gestation. Gravid uterine weight and relative kidney weights were significantly decreased and increased, respectively, at both the mid and high doses. Absolute liver weights were increased in the high dose group alone. Post-implantation losses in the high dose dams were significantly increased. The number of live fetuses per litter and average fetal weight per litter decreased significantly in the mid and high dose groups while the percentage of fetuses with malformations increased. The percentage of litters in which at least one fetus had malformations was significantly increased at all dose levels; over 96% of the high dose litters had malformations. External malformations were increased significantly among the high dose group; external malformations include cleft palate or lip, eye and abdominal muscle malformations. Increased visceral malformations were significant among the low and high doses; the increase seen at the mid dose was not significant. The most frequent visceral malformations were large vessel

anomalies. Skeletal malformations increased in a dose dependent manner with significance occurring at the mid and high doses; malformations frequently included missing or fused ribs and vertebral centra misalignment or delayed ossification. Nearly all effects were dose dependent with some effects occurring at the lowest dose of 1250 mg/kg-day.

Dose dependent increases were also seen among mice in the same study. CD-1 dams were gavaged daily with 750, 1500 or 3000 mg/kg, again on days 6 through 15 of gestation. The study was terminated on day 17 of gestation. Again, maternal signs of toxicity were not in evidence. Similarly, maternal bodyweight was significantly decreased throughout treatment and gestation in both the mid and high dose groups. Gravid uterine and absolute liver weights both decreased in the same exposure groups. Fetal bodyweight per litter was significantly decreased at all dose levels while the percent fetuses with malformations and percent litters with malformations increased greatly. External malformations, including several types of skull defects, were increased in the high dose group only. Likewise, visceral malformations were also increased; as among rats, the majority of the malformations involved the great vessels. Skeletal malformations were significantly increased at all dose levels in a dose response manner. Rib, thoracic arch and vertebral centra malformations were most prevalent. Again, ethylene glycol was effective in a dose-dependent manner; even the lowest dose in this study (i.e., 750 mg/kg-day) was effective in causing malformations (Price *et al.*, 1985).

The 1985 Price *et al.* study was reviewed in 1991 by Ryan *et al.* The review concluded that developmental effects including those seen in Price *et al.* display a clear inter-relationship between malformations and fetal weight. Malformed fetuses from ten studies showed a similar tendency to have a lower bodyweight. Ryan *et al.* highlighted the potential value of multivariate statistical models to evaluate joint effects of exposure on fetal weight and malformations.

Ethylene glycol was found to be fetotoxic but not teratogenic to rats in a whole-body exposure aerosol inhalation study. CD rats were exposed to target concentrations of 150, 1000 or 2500 $mg/m^3$ for 6 hours/day on gestation days 6 through 15. The study was terminated on day 21. All dams survived and overt signs of toxicity were not observed. Although maternal bodyweights were not affected, absolute and relative liver weights were increased in the high concentration group. Gestation parameters including fetal weight were not affected. External, visceral and skeletal malformations were not increased among treated group fetuses. However, skeletal variations which indicate fetotoxicity were significantly increased at the mid and high concentrations; these variations at the high dose included delayed ossification of the humerus and zygomatic arch. Delayed hindlimb ossification was significantly increased among fetuses in the mid exposure group; these variations were also increased in the high exposure group, but not in a significant manner. The maternal no observable adverse effect level (NOAEL) was found to be 1000 $mg/m^3$ and the developmental NOAEL was 150 $mg/m^3$. Ethylene glycol on the fur of exposed animals was analyzed as the animals were observed grooming nearly continuously during and immediately after daily exposure. The researchers estimate that 65 to 95% of the internal dose may have been due to ingestion through cleaning activities and to skin absorption. At the target inhalation dose of 2500 $mg/m^3$, the internal dose may have been as high as 620 mg/kg-day (Tyl *et al.*, 1995a).

In the same whole-body exposure aerosol inhalation study, ethylene glycol was found to be teratogenic to mice. CD-1 mice were exposed to target concentrations of 150, 1000 or 2500 $mg/m^3$ for 6 hours/day on gestation days 6 through 15. Sacrifices occurred on day 18. All dams survived. Maternal bodyweights were significantly decreased on gestation days 12

through 18 in the high exposure group. Likewise, maternal weight gain was significantly decreased in the mid exposure group during treatment and in the high exposure group during and after treatment, as compared to water aerosol exposed controls. Gravid uterine weight decreases in both of these exposure groups accounted for much of the lacking weight gain. Nonviable implants per litter were increased among both the mid and high dose groups; late resorptions were also increased. Subsequently, the percentage of live fetuses per litter were decreased in these dose groups. Fetal bodyweights were decreased in a highly significant manner at both 1000 and 2500 mg/m$^3$. External malformations were significantly increased at both of these exposure levels in a dose-dependent manner; malformations included cleft palate, exencephaly (i.e., skeletal malformation in which the brain is exposed) and protruding tongue. Visceral malformations, mainly of the brain and face, were also increased significantly in the mid and high exposure fetuses. Several skeletal malformations occurred in the mid dose group with significant frequency; numerous skeletal malformations were present in the high dose group. Malformations common to both groups include vertebral arch deformities, fused ribs and facial-cranial deformities. Skeletal variations including extra ribs and delayed cranial-facial ossification were found at the low dose. Other variations seen in the mid and high exposure groups, in a dose-dependent manner, included short ribs and delayed ossification of the vertebral centra, fore- and hindlimb and sternebrae; a few visceral and external variations were also noted. The maternal NOAEL was found to be 150 mg/m$^3$ and the developmental lowest observable adverse effect level (LOAEL) was also 150 mg/m$^3$. Ethylene glycol on the fur of exposed animals was again analyzed. The researchers estimate that 65 to 95% of the internal dose may have been due to skin absorption and ingestion through cleaning activities. At the target inhalation dose of 2500 mg/m$^3$, the internal dose in mice may have been as high as 910 mg/kg-day (Tyl *et al.*, 1995a).

The results of the previous study were confirmed with a comparison study of whole-body and nose-only aerosolized ethylene glycol exposure in mice. CD-1 dams were again exposed for six hours daily on days 6 through 15 of gestation. Nose-only groups were exposed to target concentrations of 500, 1000 or 2500 mg ethylene glycol/m$^3$; the whole-body group was exposed to a target aerosol level of 2100 mg/m$^3$. The study was terminated on day 18 of gestation. Whole-body exposure resulted in decreased maternal bodyweights from day 9 through 18. Gravid uterine weights were also significantly decreased. Nonviable implants per litter were significantly increased, due mostly to the increased prevalence of late resorptions. The percentage of live fetuses per litter were subsequently decreased, as were fetal bodyweights. External and visceral malformations were not increased. However, skeletal malformations including vertebral malformations and fused and extra ribs were significantly increased as compared to water aerosol exposed controls. Skeletal variations were also prevalent; variations included delayed ossification of vertebrae and sternebrae and extra bilateral 14th ribs. Nose-only exposure did not affect maternal bodyweights; relative kidney weights were increased in the mid dose group and both relative and absolute kidney weights were increased in the high dose group. Gestational parameters were not affected but fetal weights were decreased among high exposure group fetuses. Nose-only exposure caused no significant increases in visceral or external malformations; total skeletal malformations were not increased but the incidence of fused ribs in the high dose group was significant. Skeletal variations in the 2500 mg/m$^3$ fetuses were limited to significantly increased incidences of extra unilateral 14th ribs and delayed ossification of forelimb phalanges. Ethylene glycol isolated from the fur of whole-body exposed animals was over 4 times the ethylene glycol from 2500 mg/m$^3$ nose-only exposed animals. The nose-only maternal NOAEL was 500 mg/m$^3$ and the developmental NOAEL was 1000 mg/m$^3$ (Tyl *et al.*, 1995b).

Cutaneous ethylene glycol exposure was studied by the same group of researchers. CD-1 mice were exposed to 12.5, 50 or 100% ethylene glycol daily (approximately 404, 1677 or 3549 mg/kg-day) on gestation days 6 through 15. The exposure site was clipped and occluded; the mice were restrained during the six hours of exposure daily. The study was terminated on day 18. As a positive control, an additional group of mice was gavaged daily with 3000 mg ethylene glycol/kg bodyweight on days 6 through 15. Gavage exposure resulted in maternal death (27%) and significantly increased maternal weight gain. While kidney weights were normal, evidence of significant tubular nephrosis and cell degeneration was found. Although gestational parameters remained normal, fetal bodyweights were significantly decreased. There was no increase of external malformation of the fetuses. Visceral malformations, however, were significantly increased as were skeletal malformations. Skeletal malformations included vertebral defects and short or fused ribs. Multiple skeletal variations, primarily extra ribs and delayed ossification of vertebral centra, paws and sternebrae, were found in the gavage exposed fetuses. Cutaneous exposure to ethylene glycol did not result in maternal death but did cause increased maternal weight gain in the high dose group. Organ weights were normal and kidney lesions were not found. Fetal bodyweights were not affected and malformations (i.e., external, visceral and skeletal) were not increased. Skeletal variations were limited to delayed ossification of the parietal skull bones and of hindlimb phalanges. Maternal and fetal responses to 100% ethylene glycol were minimal; the authors reported this level as the cutaneous NOAEL (Tyl *et al.*, 1995c).

Ethylene glycol was tested in an *in vivo* short-term developmental assay using CD-1 mice. In a preliminary pilot study, non-pregnant mice were gavaged daily for eight days and then observed for an additional eight days to determine the estimated $LD_{10}$. This level was then used in the developmental assay. Dams were dosed with 11090 mg ethylene glycol/kg bodyweight daily on gestation days 7 through 14. The study terminated on postnatal day 3. Ethylene glycol caused 10% maternal mortality (5/50) and significantly decreased viable litters (41%). Dead pups per litter were significantly increased while number of live pups decreased. Postnatal survival was decreased to 40%. Pup birth weight and weight gain during the first three days was significantly decreased as compared to water exposed controls. Ethylene glycol was ranked as a high priority chemical for further developmental testing (Schuler *et al.*, 1984).

Ethylene glycol was used in an evaluation of a short-term reproductive/developmental screening assay in mice. Male and female Swiss Crl:CD-1 mice were mated prior to ethylene glycol exposure. These females were then gavaged with 250, 700 or 2500 mg ethylene glycol/kg bodyweight on gestation days 8 through 14. This portion of the study was terminated on postnatal day 4. Concurrently, the males were exposed for 18 days while a second group of females began a 20 day exposure to these same concentrations. Males and females were cohabited for five days beginning on the eighth day of exposure. The second group of females were sacrificed 24 hours post-exposure. Males were terminated on the last day of exposure. Male mice did not show overt signs of toxicity. Testicular weight, epididymal weight, sperm count and sperm motility were not affected at any dose level. No treatment related lesions were observed during histological exam. The second group of females, exposed during mating and sacrificed during gestation, also showed no outward signs of toxicity. High dose dams had significantly fewer live implants and, subsequently, more dead implants than controls; two of six litters were totally resorbed. Total numbers of implants were not affected. The first group of females, exposed during gestation and allowed to litter, were found to have similar numbers of total implants, regardless of treatment level. Pups, however, were found to have significantly decreased postnatal bodyweights (up to four days of age) when dams were exposed to

ethylene glycol at the high dose. As male parameters and the number of implants in either female group were not affected, ethylene glycol is not a likely reproductive toxicant. However, development was affected as seen in decreased viable pups and decreased postnatal weights. This screening assay correctly identified (based on literature reports) ethylene glycol as a developmental toxicant but would not identify a toxicant whose effects were only visible upon dissection (Harris *et al.*, 1992).

Ethylene glycol was assessed in a mouse two generation continuous breeding protocol study by Lamb *et al.* (1985). In a preliminary pilot study, CD-1 mice were treated for 5 days with 0.25, 0.5, 1.0, 2.5 or 5.0% ethylene glycol in the drinking water. These levels were estimated to deliver 400, 800 or 1600 mg/kg per day, respectively (Carney, 1994). Lethality occurred at both 2.5 and 5.0% levels. The continuous breeding protocol was performed using 0.25, 0.5 or 1.0% ethylene glycol. Male and female mice were exposed for seven days prior to mating. Mice were paired within the same dose level and were cohabited for 98 days (14 weeks). Litters born within this period were immediately sacrificed. After the cohabitation period, males and females were separated; resulting litters after this point were designated $F_1$. Exposure continued throughout mating, gestation and lactation. $F_1$ offspring were maintained at the same level as their parents. Bodyweights and water consumption were not affected by ethylene glycol treatment. Two females from the 0.5% group died; oxalate crystals formed from ethylene glycol were the likely cause. Most breeding pairs had four to five litters during the continuous breeding period. The high dose group had significantly fewer litters per pair, fewer live pups per litter and decreased mean pup weights, as compared to pure water controls. To finish the protocol, $F_1$ high dose and controls were mated with non-siblings from the same exposure group. Exposure at 1.0% continued for treatment group $F_1$ mice. Decreases in fertility and pup weight were not significant. Some $F_2$ pups had shorter snouts with wide-set eyes; dissection revealed shortened frontal, nasal and parietal bones with fused ribs, twisting or abnormal vertebrae and abnormal or missing sternebrae. $F_2$ controls and $F_1$ treated groups had no defects of this nature. Such subtle defects may not be detected in a single mating trial. The authors speculated that 1% of ethylene glycol which is metabolized to oxalic acid, known for chelating calcium, may result in hypocalcemia, causing bone development anomalies (Lamb *et al.*, 1993).

This study was repeated by Gulati and Lamb in 1986. Mice were dosed with 0.5, 1.0 or 1.5% ethylene glycol. Facial and axial skeletal defects were seen in the 1.0 and 1.5% dose groups (Carney, 1994).

Maternal and developmental oral NOAELs for mice were found to be 1500 and 150 mg/kg-day, respectively, in a 1989 Tyl study reviewed by Carney (1994). CD-1 dams were gavaged with 50, 150 , 500 or 1500 mg ethylene glycol/kg bodyweight on days 6 through 15 of gestation. Maternal toxicity was not found at any dose level. The high dose resulted in decreased fetal bodyweights and skeletal deformities; malformations included fused or extra ribs, fused vertebral arches and poorly ossified vertebral centra. Extra 14th ribs were the only developmental variations produced by the 500 mg/kg-day dose. Fetal effects were not found among the 50 and 150 mg/kg-day dose groups.

Ethylene glycol toxicity was also explored in New Zealand White rabbits. Artificially inseminated does were gavaged daily with 100, 500, 1000 or 2000 mg ethylene glycol/kg bodyweight on gestation days 6 through 19. The study was terminated on day 30. The high dose resulted in 42.1% mortality, early deliveries and a single spontaneous abortion on day 20. Deaths were

attributed to renal failure; intraluminal oxalate crystals, tubular dilation with degeneration and epithelial necrosis were observed. Pregnancy rates among the high dose group were lower; significance was not discussed. Maternal bodyweights, water consumption and organ weights were not significantly affected, although absolute kidney weights in the high dose group were slightly increased. Fetal resorptions, weights and malformations or variations were not affected by ethylene glycol treatment. The maternal NOAEL was 1000 mg/kg-day and the developmental NOAEL was 2000 mg/kg-day. Pregnant does were found to be more susceptible to ethylene glycol toxicity than mice or rats; however, mouse and rat fetuses have been found to be extremely susceptible as compared to rabbit fetuses (Tyl *et al.*, 1993).

Ethylene Glycol *In Vitro*/Screening Studies

The direct action of ethylene glycol on embryos was assessed using rat whole embryo culture. Whole embryo culture utilizes embryos complete with yolk sacs and associated placental tissue. Metabolism of the compound is negligible as whole embryo units lack significant ADH or ALDH activity. Rat embryos were harvested at 10.5 days of gestation. Incubation at 30 µl ethylene glycol/ml medium (531 mM) for 48 hours was 100% lethal. Incubation at this dose or at 40 µl/ml (710 mM) for either the first or second 8 hour period was tolerated; normal medium was used for the last 40 hours (or first 8 and last 32 hours) of incubation. Either dose inhibited embryonic development; DNA and protein contents were decreased as were the crown-rump lengths of the embryos. Abnormalities, including lack of yolk sac circulation, hind limb buds and otic and optic rudimentary systems, were identified; hypoplasia of the telencephalon was also found (Grafton and Hansen, 1987 as cited by Carney, 1994).

Levels embryotoxic to rat whole embryo cultures were found to be significantly higher than human blood levels in fatal ethylene glycol poisonings. Rat embryos were explanted on day 10.5 of gestation and incubated in ethylene glycol at concentrations of 5.0 to 25.0 µl/ml medium for 40 hours. Ethylene glycol was embryotoxic in a dose dependent manner; the lowest effective dose was 14.2 µl/ml at which the embryo protein content was reduced. Although crown-rump lengths were normal, embryos also appeared edematous. Culture concentrations are frequently compared with human serum levels. Fatal human ethylene glycol poisonings result in serum ranges of 0.1 to 2.7 µl ethylene glycol/ml blood (Brown-Woodman *et al.*, 1994a).

The direct effect of ethylene glycol on whole embryo culture was compared with the effect of glycolic acid in a study by Carney *et al.* (1995). Rat embryos were explanted at 10.5 days of gestation and incubated for 46 hours in medium with varying concentrations of ethylene glycol or glycolic acid. Concentrations of ethylene glycol in medium were 0.5, 2.5, 12.5, 25 or 50 mM; pH remained at 7.5. These same concentrations of glycolic acid were added to medium while the pH ranged from 7.5 to 4.3. Concentrations were based on maternal peak blood levels found in the literature; 11 mM ethylene glycol and less than 2 mM glycolic acid were found in dams at NOEL dose levels. Similarly, 21 mM ethylene glycol with 2 mM glycolic acid and 43 mM ethylene glycol with 13 mM glycolic acid were found at maternal NOEL and developmentally toxic concentrations, respectively. In this study, levels of ethylene glycol up to 50 mM with glycolic acid levels of 2.5 mM resulted in no toxicity. Concentrations of 12.5 mM glycolic acid resulted in decreased embryo growth and protein content as well as dysmorphogenesis in the cranial-facial region. Higher concentrations of glycolic acid were lethal. Subsequently, another study was performed in which embryos were cultured in 12.5 mM

glycolic acid at pH 6.7, 12.5 mM sodium glycolate at pH 7.4 or in control media at pH 6.7 or 7.4. Growth and development were adversely affected among embryos incubated in sodium glycolate and pH 6.7 media. Glycolic acid, however, was more effective in decreasing growth and development. Both studies indicate that ethylene glycol toxicity is linked to high levels of glycolic acid, which causes metabolic acidosis and intrinsic developmental effects.

In an evaluation of the chick embryo toxicity study utilizing intra-yolk injection, the effect of ethylene glycol was assessed. White Leghorn Eggs were injected with 0.05 or 0.10 ml neat ethylene glycol. The eggs were then incubated until hatched. Ethylene glycol decreased the percent of eggs hatched in a dose dependent manner (i.e., 65, 80 and 95% hatched from 0.10 ml, 0.05 ml or control groups, respectively) (McLaughlin *et al.*, 1963). Intra-yolk injection was rated poorly as a method of comparative embryotoxicity and teratogenicity in a study by Walker (1967). Movement in liquid yolk was found to be related to the density of the material. Movement in intact yolks was similar; ethylene glycol, which is nearly the same density as the yolk, stayed near the center of the yolk and formed semisolid coagula. Ethylene glycol injected (0.05 or 0.10 ml) into unincubated eggs resulted in a 72% survival rate as compared to injection into three day incubated eggs, of which 100% survived. Other tested substances with smaller densities rose and tended to cause high mortality. An additional study with malathion demonstrated that the vehicle in which a substance is dissolved can affect outcome (i.e., one vehicle may cause teratogenicity while another may cause fetotoxicity when administered with a test substance, but administered alone as controls may not have any effect). The dependence on vehicle is lessened if three day embryos are used instead of unincubated eggs.

Ethylene glycol near yolk injection was evaluated in a 1984 Ameenuddin and Sunde study. New Hampshire x Single Comb White Leghorn fertile unincubated eggs were injected near the yolk with 0.10 ml ethylene glycol per egg. Percent hatchability was depressed in a highly significant ($p<0.01$) manner as compared to sham-injected and corn oil controls (47.6, 82.1 and 81.7%, respectively). In a subsequent study, eggs were injected with 0.05, 0.10 or 0.15 ml. The low dose was well tolerated and only slightly, but significantly, decreased hatchability. Both mid and high dose levels had similar extreme effects on hatchability as before.

Ethylene glycol was found to disrupt development only at concentrations near adult toxic exposure levels in the hydra developmental assay. Adult *Hydra attenuata* and "artificial hydra embryos" (i.e., reaggregated adult cells) were first exposed to ethylene glycol diluted in water at whole log concentrations ranging from $10^{-3}$ through $10^{3}$ ml/l. The resulting NOAEL and LOAEL for both adults and developmental stages were tested again along with the 1/10 log concentrations between to derive the MEC. The adult (A) and developmental (D) MECs for ethylene glycol were 50.0 and 30.0 ml/l, respectively, yielding an A/D ratio of 1.7. Since this ratio was near 1, ethylene glycol is therefore developmentally disruptive near adult toxicity levels (Johnson *et al.*, 1984). In a 1988 review, Johnson *et al.* compared the results of the hydra assay to a mammalian A/D ratio derived from literature values. Using rat and mouse study results (Price *et al.*, 1985), the mammalian A/D ratio was calculated to be 2.0, supporting the hydra assay outcome.

## Propylene Glycol

Propylene glycol may be used as an aircraft deicer/anti-icer or as a runway deicer. Like ethylene glycol, propylene glycol is effective at low temperatures and melts through existing ice

and snow. Propylene has a higher BOD than ethylene glycol (Mericas and Wagoner, 1996) but is favored over ethylene glycol due to its lesser mammalian toxicity. The U.S. Air Force is phasing out ethylene glycol because of potential human toxicity resulting from airfield runoff. Propylene glycol is the substitution of choice (McKenna *et al.*, 1996).

Evidence of propylene glycol activity on the endocrine system is equivocal. Although this compound did not affect adrenal hormone production in rats, it caused increased ovulation in ewes. Some *in vivo* developmental studies in rodents resulted in fetotoxicity and teratogenicity, while others reported no effects from ethylene glycol administration. Use of propylene glycol as a cryoprotectant caused some embryonic damage that could result in developmental disruption of implanted embryos. *In vitro* developmental studies using mouse embryos, chicken eggs and *Hydra* revealed no adverse effects.

Propylene Glycol Toxicity Studies

Sprague-Dawley rats were used in a nose-only subchronic 90-day study of propylene glycol aerosol. Male and female rats were exposed to 0.16, 1.0 or 2.2 mg propylene glycol/l air for 6 hours daily, 5 days/week for 13 weeks. Differences in bodyweights of exposed groups of males were found to be not significant. High dose females had significantly decreased bodyweights beginning on day 50 and persisting through the end of the study. Mid concentration females had significantly lower weights starting on day 64. These weight effects correlated with decreased food consumption beginning on days 43 and 50 for the high and mid exposure group females, respectively. There was, however, no effect on absolute or relative weights of the adrenal glands, testes, prostate, uterus or ovaries. No treatment related effects were seen in respiratory rate, tidal volume or minute volume. Statistically relevant differences occurred in some hematological parameters, serum enzyme activities and lung, spleen, liver and kidney weights; these differences were not consistent and did not show dose-response trends. Mid and high exposure group animals displayed increased goblet cells and increased mucin within goblet cells. Nasal hemorrhage and ocular discharge frequently occurred, likely due to dehydration of these mucosal tissues (Suber *et al.*, 1989).

Oral toxicity feeding studies in CD rats by Gaunt *et al.* (1972) were reviewed in an article by the Cosmetic Ingredient Review Expert Panel (1994). Male and female rats were exposed to 50,000 ppm propylene glycol in the diet for 15 weeks in the subchronic study. The daily dose was approximated at 2.5 g propylene glycol/kg bodyweight. No significant treatment related lesions or weight changes were seen in the ovaries, testes, adrenals or pituitary glands. Additionally, no differences were seen among brain, heart, spleen, liver or kidney tissues as compared to controls. The authors also performed a two year chronic study. Rats were again fed propylene glycol in the diet; the dose levels were 6250, 12,500, 25,000 or 50,000 ppm. Animals were sacrificed at 104 weeks; organ weights, including the adrenal glands, were not different from controls. Bodyweights also were not affected. Lesions were not treatment related. A high number of mammary gland fibroadenomas were found among both treated and control female rats; these neoplasms are known to occur among aging CD rats. No carcinogenic potential for propylene glycol at doses up to 50,000 ppm in the diet was found.

## Propylene Glycol Neuroendocrine Studies

In a report on the safety of propylene glycol, the Cosmetic Ingredient Review Expert Panel (1994) detailed a study on the ability of propylene glycol to induce adrenal steroidogenesis in the rat. Male Fischer rats were administered 0.4 mg/ml 7% (v/v) propylene glycol intravenously. After 30 minutes, 0.2 μg ACTH was also administered; ACTH caused aldosterone to be released into the serum. Serum samples taken 30, 60 or 90 minutes post-ACTH administration demonstrated that propylene glycol did not have any significant effects on adrenal hormone production (Fish *et al.*, 1988).

## Propylene Glycol Reproduction Studies

The use of propylene glycol as a vehicle for FSH has been found to increase ovulation rates in ewes, although the response in individuals has been highly variable. Manchega ewes were injected intra-muscularly with 5 or 10 mg FSH in saline or propylene glycol on different days of estrus. Additionally, 100 μg cloprostenol was injected to induce the estrus cycle. Peritoneoscopy was performed 7 to 12 days later in order to count corpus lutea. FSH in propylene glycol injected on day 13 of estrus along with cloprostenol induced increased mean numbers of corpus lutea in ewes as compared to FSH in saline. However, variance between individuals also increased as the mean number of corpus lutea increased. Propylene glycol appears to be a slow-releasing vehicle and seems to allow the normally short-lived FSH (110 minutes in saline) to retain its biological reactivity. Alternatively, propylene glycol may change enzyme activity in the liver. FSH in saline and propylene glycol combined, injected at different sites, produced increased corpus lutea as compared to saline alone; however, the number of corpus lutea were decreased as compared to FSH administered in propylene glycol alone (Lopez-Sebastian *et al.*, 1993).

## Propylene Glycol Developmental Studies

Propylene glycol was used as a solvent in a 1977 teratogenicity study of 4-nitroquinoline-1-oxide by Nomura. Solvent controls of both propylene glycol and water were tested. Pregnant ICR/Jcl mice were exposed via subcutaneous injection to 0.01 ml 50% propylene glycol aqueous solution/g bodyweight or 0.01 ml water/g bodyweight on days 9, 10 or 11 of gestation. Propylene glycol did not increase the number of pre- or post-implantation deaths or decrease the number of live pups, as compared to water or untreated controls. The number of fetuses with malformations was slightly increased over water and untreated groups; pups exposed to 15 μg 4-nitroquinoline-1-oxide/g on day 10 of gestation displayed a similar occurrence rate of malformation which was not found to be statistically significant. Malformations induced by propylene glycol included isolated occurrences of cleft palate and polydactyly (i.e., excess digits), as well as a few occurrences of open eyelid. In a subsequent study, 11 day embryos were injected with 0.005 ml 10% propylene glycol by microsyringe through the uterine wall and into the amniotic cavity. No other control groups were tested. Pre-implantation deaths were not increased as compared to maternal subcutaneous exposure results. However, post-implantation deaths increased from 3.4 to 23.8% and live fetuses decreased from 96.6% to 76.2% (subcutaneous exposure compared to intra-amniotic exposure, respectively). Malformations increased from 2.2 to 6.3%; anomalies included tail and leg defects. Since

propylene glycol served as the solvent in these studies, statistical analyses were not available on its effects. It does appear that propylene glycol has some teratogenic potential at these levels.

Propylene glycol was found to be non-teratogenic in a study initially reported by Kavlock *et al.* (1987). CD-1 mice were dosed with 10,000 ppm orally on days 8 through 12 of gestation. No maternal deaths were seen. Fertility rates were not different from controls and resorptions were not observed. Average litter size, pup birth weights and weight gain were not adversely affected (Cosmetic Ingredient Review Expert Panel, 1994.)

In a continuous breeding study, male and female COBS Crl:CD-1 (ICR)BR Swiss albino mice were exposed to propylene glycol via drinking water or diet. For 7 days prior to mating, mice were exposed to 1.0, 2.5 or 5.0% propylene glycol (1.82, 4.80 or 10.10 g/kg bodyweight, respectively). Animals were then paired and treated for 98 days. No significant changes in live pup weights were noted among the offspring of any of the treated mice. Subsequently, these $F_1$ mice were co-mated. $F_1$ mice had been exposed throughout gestation, nursing, weaning and breeding at approximately 74 days of age. No significant changes were seen in the mating index, fertility index, number and proportion of live pups. No difference in sex ratio was seen among $F_2$ pups (Morrissey *et al.*, 1989 as cited by Cosmetic Ingredient Review Expert Panel, 1994.)

Propylene Glycol *In Vitro*/Screening Studies

Propylene glycol (i.e., 1,2-propanediol) is a common cryoprotectant for mammalian zygotes preserved by rapid freezing. Damien *et al.* (1990) examined the effects of propylene glycol on the pH and development of mouse zygotes. $B_6D_2F_1$ zygotes were collected 22 to 24 hours after fertilization. Zygotes were pre-incubated with 0.5 μM acridine orange, a dye which fluoresces bright green-yellow at physiological pH (i.e., 7.4) and dull red at lower pH levels. Zygotes were then perifused for 20 minutes in 0.18, 0.36 or 1.0 mol propylene glycol/minute; this slow introduction of propylene glycol into the medium prevents the immediate cell volume loss seen if zygotes are placed directly into propylene glycol solution. Volume changes were noted throughout the perifusion duration. The low and mid exposure levels had no significant effect on zygote volume while the high exposure level significantly decreased zygote volume 70% within the first five minutes of perifusion. Control zygotes perifused with physiological saline maintained fluorescence through perfusion. Propylene glycol perifused zygotes maintained fluorescence at five minutes. However, at 10 minutes, all high exposure zygotes had lost fluorescence while mid and low exposure zygotes lost all fluorescence at 15 and 20 minutes, respectively. To test developmental effects of propylene glycol, zygotes were perifused in 3.0 M propylene glycol for 2.5, 5, 10 or 15 minutes, transferred to regular culture medium and incubated for 24 or 96 hours. Zygotes perifused for 2.5 minutes were able to form 2-cell embryos at 24 hours but had a significantly decreased ability to form blastocytes at 96 hours. Both 2-cell and blastocyte formation were inhibited in zygotes perifused for 5, 10 or 15 minutes. Decreased pH within the cell may alter physical and electrical properties of the cell membrane. Blastocyte formation depends on epidermal growth factor which stimulates protein synthesis via receptors on the cell membrane. Since propylene glycol alters the zygotes' ability to form blastocytes, seemingly normal cryopreserved embryos transferred in the 4- or 8-cell stage may not develop, decreasing expected pregnancy rates.

Propylene glycol may also be used as a cryoprotectant for mammalian oocytes. The preserving capabilities of propylene glycol on mouse oocytes was investigated by van der Elst *et al.* (1988). Oocytes from $F_1$ hybrids (C57B1/6J females x CBAca males) were harvested 48 hours after ovulation was induced. The effect of cooling on the meiotic spindle of the oocytes was tested by exposing oocyte cultures to room temperature (i.e., 20°C) with or without 1.5 M propylene glycol for 30 or 60 minutes. Recovery was tested by raising the temperature to 37°C for 30 minutes after zygotes were removed from the 20°C propylene glycol solution. Viability of the embryos was ascertained by *in vitro* insemination with freshly harvested sperm. After 4 hours of insemination, embryos were cultured for 24 hours. Cooling to 20°C severely affected the percentage of oocytes with normal meiotic spindles in a time dependent manner. Reversibility of these effects was not complete and again was dependent on the amount of time at 20°C. The presence of propylene glycol increased the number of oocytes with normal spindles by three to five times. However, reversibility of spindle effects was low; 83-95% of oocytes had no spindle evident when warmed to 37°C. Fertilization rates were not significantly lower for propylene glycol exposed oocytes and the genetic make-up of the resulting embryos (as indicated by normal embryo mitosis) was not different from controls; spindle recurrence is indicated. No mechanistic evidence of biochemical toxicity of propylene glycol was present; passage of propylene glycol through the oocyte membrane may cause ionic differences which induce depolymerization of the spindle. The authors suggest investigation of possible resulting developmental abnormalities.

Propylene glycol effects on early mouse embryos were studied by Kowalczyk *et al.* (1994). Two-cell mouse embryos were explanted and exposed to 0.05, 0.1, 0.2, 1.0 or 2.0% propylene glycol in medium for 24 hours at 37°C. Development was observed over five days. Propylene glycol did not impair or accelerate development of the blastocyst. Subsequently, eight cell morulae were exposed to similar concentrations, again for 24 hours. Propylene glycol did not adversely affect the percentage of embryos cavitating, the time at which cavitation started or blastocoel volumes.

Evidence of propylene glycol teratogenicity was not found in an *in vitro* test utilizing mouse ovarian tumor cells. Ovarian tumor cells were labeled with [$^3$H]thymidine and suspended in various concentrations of propylene glycol. A teratogen would prevent these cells from adhering to the Concanavalin A-coated plastic of the test well; adherence was measured by the level of radioactivity of the plastic well after the test solution was removed. Propylene glycol did not inhibit attachment of the ovary tumor cells to the plastic (Braun *et al.*, 1982 as cited by Cosmetic Ingredient Review Expert Panel, 1994).

In an evaluation of the chick embryo toxicity study utilizing intra-yolk injection, the effect of propylene glycol was assessed. White Leghorn Eggs were injected with 0.05 ml neat propylene glycol. The eggs were then incubated until hatched. Propylene glycol did not effect the percent of eggs hatched (i.e., a 95% hatch rate occurred in both the treated and control groups) (McLaughlin *et al.*, 1963). Intra-yolk injection was rated poorly as a method of comparative embryotoxicity and teratogenicity in a study by Walker (1967). Movement in liquid yolk was found to be related to the density of the material. Movement in intact yolks was similar; propylene glycol, which is nearly the same density as the yolk, stayed near the center of the yolk and formed semisolid coagula. Propylene glycol injected (0.05 or 0.10 ml) into unincubated eggs had only a 67% survival rate as compared to injection into 3 day incubated eggs, of which 100% survived. Other tested substances with smaller densities rose and tended

to cause high mortality. An additional study with malathion demonstrated that the vehicle in which a substance is dissolved can affect outcome (i.e., one vehicle may cause teratogenicity while another may cause fetotoxicity when administered with a test substance, but administered alone as controls may not have any effect). The dependence on vehicle is lessened if three day embryos are used instead of unincubated eggs.

Propylene glycol near yolk injection was evaluated in a 1984 Ameenuddin and Sunde study. New Hampshire x Single Comb White Leghorn fertile unincubated eggs were injected near the yolk with 0.10 ml propylene glycol per egg. Percent hatchability was depressed in a significant ($p<0.05$) manner as compared to sham-injected and corn oil controls (67.7, 82.1 and 81.7%, respectively). In a subsequent study, eggs were injected with 0.05, 0.10 or 0.15 ml. All dose levels had similar effects on hatchability; no significant differences were found between sham-injected, corn oil and propylene glycol treated groups.

In a review of human reproductive hazards, Shane (1989) identified propylene glycol as a solvent which has been found teratogenic or embryotoxic. Shane cited a chick embryo study by Gebhardt (1968).

Propylene glycol was found to disrupt development only at concentrations near adult toxic exposure levels in the hydra developmental assay. Adult *Hydra attenuata* and "artificial hydra embryos" (i.e., reaggregated adult cells) were first exposed to propylene glycol diluted in water at whole log concentrations ranging from $10^{-3}$ through $10^{3}$ ml/l. The resulting NOAEL and LOAEL for both adult and developmental stages were tested again along with the 1/10 log concentrations between to derive the MEC. The adult (A) and developmental (D) MECs for propylene glycol were 40.0 and 30 ml/l, respectively, yielding an A/D ratio of 1.3. Since this ratio was near unity, propylene glycol was therefore determined to be developmentally disruptive at or near adult toxicity levels (Johnson *et al.*, 1984).

## Urea

Urea as a deicer is available in both solid and liquid form. The solid pill is the most common form used; liquid urea is also used as a mixture with ethylene glycol. Urea is effective down to -7°C and is relatively inexpensive. Although urea is the historic runway deicer of choice for North American airports, it is being phased out through regulatory and industry pressure due to its high nitrogenous BOD. Urea use on runways often results in a BOD tens to hundreds of times higher than the BOD allowed in surface water discharge. It is the ammonia released when urea breaks down that is responsible for the high BOD. Ammonia is also directly toxic to aquatic life (Mericas and Wagoner, 1996).

Few urea or ammonia studies were located in this literature review; however, some evidence of endocrine activity was found. Urea was not a reproductive toxicant by the Sperm Head Abnormality test. Urea was found to affect thyroid glands and thyroid hormone levels in chick embryos. Similarly, ammonia is capable of affecting different areas of the rat brain as well as insulin levels in steers. The mechanisms of these effects are not known; urea may affect the hormone system directly or cause physical changes which indirectly result in endocrine activity.

Urea Toxicity Studies

Ammonia is an expected by-product of urea in the environment. Ammonia was found to effect the amount of tubulin, the basic protein structure of cellular microtubules, produced in areas of the rat brain. Hyperammonemia was induced in male Wistar rats through the administration of 20% (w/w) ammonium acetate in the diet for 2 months. Tubulin concentrations were increased in six of eleven discreet regions of the cerebrum, including the hypothalamus, septum and hippocampus. Areas of the hippocampus were most affected, suggesting that ammonia affects the hippocampus first, which then affects the septum and hypothalamus. Two methods of ammonia action, direct effects on the hippocampus and indirect afferent stimulation of other areas, are suggested. The mechanism of afferent stimulation was not hypothesized (Minana *et al.*, 1989).

Regulatory hormone effects were measured in steers exposed to ammonia. Seven Herefords were dosed with 12 μmol ammonium chloride/kg bodyweight per minute for 240 minutes via a catheter in the right jugular vein. Saline infusions lasting 120 and 180 minutes preceded and followed exposure, respectively. Blood samples were taken every 20 minutes from the left jugular vein catheter throughout the infusion and exposure periods. The hyperammonemia induced was subclinical; no signs of toxicity (i.e., reduced intake of food, neurological symptoms, metabolic acidosis) were observed. Plasma levels of glucose increased during exposure and remained elevated post-exposure. Plasma urea, L-lactate and nonesterified fatty acids also increased while pyruvate, acetoacetate and β-hydroxybutyrate concentrations were not affected. Plasma insulin concentrations decreased during exposure but increased during the post-exposure period, as compared to pre-exposure levels. As plasma glucagon levels were not affected, molar insulin:glucagon ratios mirrored the changes in insulin levels. Plasma catecholamine levels tended to increase during exposure and post-exposure, although this increase was not significant. High degrees of variability in catecholamine levels were seen between steers and sampling times. The individual catecholamines (i.e., epinephrine, norepinephrine and dopamine) were not significantly different during or after exposure as compared to before exposure levels. The hyperglycemia induced in this study appears to result from decreased utilization of glucose during hyperammonemia. The insulin:glucagon ratio is thought to have a greater impact on regulating glucose than either hormone alone. Catecholamines appear to increase as a secondary response to the stress of hyperglycemia (Fernandez *et al.*, 1988).

Urea Reproduction Studies

Urea was evaluated in the sperm-head abnormality test (SHA) by Topham (1980). In this test, male mice ((CBA males x BALB/c females)$F_1$) were intraperitoneally administered the test substance at fractions of the $LD_{50}$ for five contiguous days. Urea was dosed at 250, 500, 1000 or 2000 mg/kg per day in physiological saline. Caudal sperm were collected and evaluated five weeks post-exposure. A positive response would include at least double the rate of SHA occurrence in the negative control group and also statistical significance at $p<0.05$. The SHA evaluation for urea was negative. The concentrations of urea used were not found to be lethal. The SHA test is useful for identification of substances which cause transmissible genetic damage by disrupting the process of differentiation of spermatozoa.

Urea *In Vitro*/Screening Studies

The chicken embryo assay was used as a model for thyroid toxicity of urea. Hybrid incubated eggs were dosed with 200, 400, 800 or 1200 mg urea per kg of egg weight. Dissolved in 0.05 ml of distilled water, urea was injected into the air sacs of the eggs on days 16, 17 and 18 of incubation. The embryos were examined on day 19. Urea increased embryo mortality in a dose-response manner with nearly 40% mortality at the high dose. Blood levels of $T_3$ hormone were increased while $T_4$ levels were decreased. Liver enzymes (e.g., AST and lactate dehydrogenase (LDH)) were slightly increased. Electron microscopic examination of the thyroid glands revealed cytoplasmic edema, mitochondrial swelling and external membrane damage in the thyrocytes. This study demonstrated urea-induced thyroid damage. Thyroid gland enlargements and disorders have been reported in ruminants exposed to urea over the long term (Mora *et al.*, 1991). In 1976, one-fifth of the U.S. production of urea was used in livestock feeds (Fleischman *et al.*, 1980).

## Sodium Formate

Sodium formate, commonly used in Europe, is an irregularly shaped crystal; this property decreases the likelihood of blowing in windy conditions. Sodium formate is more effective than urea at lower temperatures. Only 40 to 60% of the effective volume of urea is needed to achieve the same results; however, sodium formate is 1.5 times more expensive to use than urea. Sodium formate also has a significantly lower BOD than urea, sodium acetate or calcium magnesium acetate (Mericas and Wagoner, 1996).

Only one *in vivo* developmental study was found as a result of this literature review; no effects were seen as a result of gestational exposure in mice. Several *in vitro* embryo tests were available. Each reported increased embryotoxicity and dysmorphogenesis in response to sodium formate. Although the mechanisms of these changes were not hypothesized, sodium formate was found to have direct cellular effects in another *in vitro* assay. When toxicity is displayed through *in vitro* testing, further whole animal studies are recommended. Outside of developmental toxicity, additional endocrine related studies were not located in this review.

Sodium Formate Developmental Studies

Dorman *et al.* (1995) explored the role of formate, an oxidative metabolite of methanol, in methanol teratogenicity. CD-1 dams were exposed on day 8 of gestation (i.e., the time at which neurulation occurs) to 750 mg/kg sodium formate via gavage. A pilot study using 25, 250, 500 or 750 mg/kg sodium formate found that the high dose best approximated blood formate levels found after a 10,000 ppm 6 hour exposure to methanol. The embryos were examined on day 10 or 18 of gestation. Incidence of open anterior neural tubes found on day 10 was not significantly increased as compared to incidence among controls. No open neural tubes were found on day 18 of gestation. A subsequent *in vitro* study was performed using explanted mouse embryos. Gestation day 8 embryos were cultured with 4, 8, 12, 20 or 40 mM sodium formate for 24 hours. Concentration dependent dysmorphogenic effects were observed. Incidence of severely abnormal neural seams, asymmetrical prosencephalons (i.e., forebrain which divides into diencephalon and telencephalon) and branchial arch hypoplasia (i.e., underdevelopment of pharynx region) became significant only at the 40 mM dose level. The

neural folds of some treated embryos were less elevated and more swollen than control embryos. A dose dependent decrease in yolk sac diameter and embryo crown-rump length was also seen. Additionally, rotation was delayed among sodium formate treated embryos. Blood formate levels of 7 to 10 mM have been reported for humans with neuro-ocular toxicity from methanol ingestion; sodium formate appears to be capable of dysmorphogenic effects at this level. The exencephaly (i.e., skull deformity in which the brain is exposed or extruding) seen in methanol teratogenesis can not be attributed to formate production.

Sodium Formate *In Vitro*/Screening Studies

The visceral yolk sac of embryos is know to have metabolic capabilities. In order to evaluate the direct effects of methanol and two of its metabolites, sodium formate and formaldehyde, microinjection into the amniotic fluid of rat embryos was used. Rat fetuses were explanted on day 10 of gestation and injected with minute quantities of parent compound or metabolites. Sodium formate decreased embryo viability at 5.0 µg. Based on an intra-amniotic fluid volume of 500 nl, the calculated toxic concentration for sodium formate was 10 mg/ml. The toxic concentration for methanol itself was 350 mg/ml, indicating that methanol undergoes significant visceral yolk sac metabolism to produce toxicity in whole embryo cultures. Sodium formate caused toxicity at similar concentrations used in whole embryo cultures (Contreras and Harris, 1995).

Sodium formate was found be intrinsically toxic to rat whole embryo culture, although decreasing pH may have an additive effect. Sprague-Dawley rat embryos were explanted at 9.5 days of gestation and incubated in 5 ml culture media. Sodium formate was present in concentrations of 0.2, 0.4, 0.8, 1.2 or 1.6 mg/ml medium. pH of the cultures was altered with 0.2 N HCl; median pH values were 8.13, 7.75, 7.00, 6.50 or 6.00. After 48 hours incubation, crown-rump length, head length, somite numbers, developmental scores and protein concentrations were all significantly decreased at 1.6 mg sodium acetate/ml, regardless of pH. However, at lower pH levels (e.g., 6.5), embryotoxicity was significantly increased at 0.4 and 0.8 mg sodium acetate/ml. Yolk sac diameter was significantly decreased at all pH levels with 1.6 mg formate/ml medium. Embryonic abnormalities were significantly increased at 1.6 mg/ml or 1.2 mg/ml with a pH of 7.0 or lower. Abnormalities included delayed axial rotation, abnormal neural seams and open neural tubes (i.e., incomplete fusion of rhombencephalon, mesencephalon or telencephalon). Sodium formate was found to be a developmental toxicant whose effects may be enhanced by decreased pH levels (Andrews *et al.*, 1993).

Methanol may be metabolized to formate or formic acid; both were tested on rat and mice embryos in a 1995 study by Andrews *et al.* Sprague-Dawley rat embryos were explanted on day 9 of gestation and cultured in 5 ml media with sodium formate or formic acid for 24 or 48 hours. Sodium formate exposure levels were 0.2, 0.4, 0.8, 1.2, 1.6 or 2.0 mg/ml culture media (i.e., 2.95, 5.9, 11.8, 17.7, 23.5 or 29.4 mM). Formic acid concentrations were 0.14, 0.27, 0.54, 0.81 or 1.08 mg/ml culture media (2.95, 5.9, 11.8, 17.7 or 23.5 mM). Embryos exposed for 24 hours to sodium formate had significant dose-dependent decreases in yolk sac diameter, crown-rump and head length, somite number and developmental scores. While embryolethality was not increased, CNS effects such as open neuropores and erratic neural seams were increased as compared to controls. Rotation defects were also observed as well as decreased DNA and protein levels. Exposure for 48 hours increased embryotoxic effects. Embryolethality was observed at the highest dose level; rotational and tail abnormalities were observed at the

lowest. Formic acid exposure for 24 hours resulted in significant dose-dependent embryotoxicity (i.e., yolk sac diameter, etc.) and increased embryolethality at the high dose (i.e., 1.08 mg/ml or 23.5 mM). After 48 hours incubation, the high dose was 100% lethal. Increased embryolethality and anomalies (e.g., rotational defects, enlarged maxillary processes, failed closure of anterior and posterior neuropores) occurred at 0.81 mg/ml (17.6 mM). Sodium formate and formic acid were found to be embryotoxic and dysmorphogenic in a dose dependent manner. Both sodium formate and formic acid were found to be more toxic to the developing embryo than methanol, on a molar scale.

CD-1 mice embryos were also used in the 1995 Andrews *et al.* study. Embryos were explanted on day 8 of gestation and exposed for 24 hours. Sodium formate levels of 0.4, 0.8, 1.6, 2.0 or 3.0 mg/ml culture media (5.9, 11.8, 23.5, 29.4 or 44.1 mM) or formic acid levels of 0.27, 0.54, 0.81, 1.6, 2.0 mg/ml culture media (5.9, 11.8, 17.6, 34.8 or 44 mM) were used. Significant dose response decreases in yolk sac diameter, crown-rump length, head length, somite number and developmental score were seen among mice embryos after 24 hours incubation in sodium formate. Embryolethality was not increased by sodium formate exposure. However, anomalies including open neuropores (both anterior and posterior) and erratic neural seams were evident in a dose dependent manner. DNA and protein concentrations decreased with increasing concentration of sodium formate; their ratio was not affected. Anomalies observed only at the high dose of formate included enlarged maxillary processes and pericardium as well as delayed heart development. Formic acid exposure produced similar effects to a greater degree. At the high dose (i.e., 2.0 mg/ml or 40 mM), embryolethality was significantly increased; 100% malformations occurred among survivors. Again, sodium formate and formic acid were found to be embryotoxic and dysmorphogenic in a dose dependent manner; both sodium formate and formic acid were found to be more toxic to the developing embryo than methanol, on a molar scale. Rat and mice embryos were not found to respond in a significantly different fashion.

While assessing the effects of 2-methoxyacetic acid on cultured mouse embryos, Stedman and Welsch (1989) evaluated the attenuative effects of sodium formate or acetate. CD-1 mice embryos were harvested on gestation day 11 and exposed to 1 mM sodium formate for 5 hours. [$^3$H]Thymidine was added and the embryos were cultured for an additional hour; the rate of DNA synthesis was measured by the uptake of this labeled amino acid. The amount of DNA within exposed embryos was not different from control embryos incubated in pure media and [$^3$H]thymidine. The rate of DNA synthesis in formate exposed embryos was also not statistically different. Exposure to 24 mM 2-methoxyacetic acid, the teratogenic metabolite of 2-methoxyethanol, resulted in a 50% reduction in DNA synthesis in the treated embryos. Embryos incubated with 2-methoxyacetic acid and sodium formate had normal levels of DNA synthesis. Sodium formate was found to attenuate teratogenicity of 2-methoxyacetic acid at one-fifth the effective concentration of sodium acetate (i.e., 1 mM sodium formate had the same effect as 5 mM sodium acetate).

The effect of sodium formate on primary cultures of mouse cerebrocortical neural cells was investigated by Dorman *et al.* (1993). Cerebrocortical cells, including neurons and glial cells, were isolated from gestation day 15 CD-1 mouse embryos. Mature cultures of equal cell density after 7 to 15 days were pre-incubated for 8 hours in carbon-14-labeled adenine nucleotides. Cultures were then exposed to sodium formate concentrations ranging from 0 to 240 mM for 8 hours at pH levels ranging from 6.0 to 7.6. Cell cytotoxicity, measured through histopathology and membrane integrity changes (i.e., release of LDH or arginine leakage), was dose-dependent. Estimated 50% LDH leakage occurred at 45 mM. Formate concentrations of

20 to 60 mM appeared to be neuronotoxic, targeting large polygonal neurons. Concentrations of sodium formate greater than 120 mM were non-specifically cytotoxic. Mitochondrial metabolic activity was significantly decreased at only 20 mM; intracellular ATP concentrations were also significantly decreased at 20 to 40 mM sodium formate. Formate appeared to inhibit mitochondrial function which in turn decreased ATP, resulting in neuron degradation.

## Sodium Acetate

Sodium acetate is a fairly recent addition to the Federal Aviation Administration (FAA) runway deicer list. It is available in solid form and may need to be used in conjunction with a liquid deicer (i.e., propylene or ethylene glycol) as a pre-wetting agent, allowing the solid to adhere to pavement during windy conditions. Sodium acetate reacts with ice faster than urea and is effective at two-thirds the volume of urea needed, but is currently three times as expensive as urea to use (Mericas and Wagoner, 1996).

Sodium acetate does appear to be an endocrine active compound. First, sodium acetate can act as a metabolic precursor of cholesterol and steroid hormones. Second, sodium acetate affected thyroid function in a rat toxicity study. Third, *in vivo* developmental studies showed decreased fertility after gestational exposure of rodents to sodium acetate. However, sodium acetate has been used as the control compound for *in vivo* and *in vitro* lead acetate studies with apparently little adverse effect. *In vitro* developmental studies using rodent and chick embryos did not identify sodium acetate as a teratogen. Sodium acetate even attenuated the teratogenic effects of 2-methoxyacetic acid. Mechanisms of developmental toxicity were not proposed, could be related to the sodium ion and may be separate from the synthesis of hormones and precursors.

### Sodium Acetate Kinetics Studies

Synthesis of cholesterol and steroids was examined in human fetal liver tissue using carbon-14 labeled sodium acetate. Livers were obtained from three human fetuses aborted during gestational weeks 16 through 20. Livers were minced and incubated with 10 mCi of 55.4 mCi/mmol sodium acetate solution for 5 hours in the presence or absence of cofactors (i.e., ATP, NADP, DPN, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and nicotinamide). Production of labeled cholesterol, pregnenolone and dehydroepiandrosterone was measured. Human fetal liver preparation was found to produce relatively large quantities of cholesterol (0.95 to 3.1% incorporation of labeled material) from $^{14}$C-sodium acetate. Radio-labeled steroids were not found. Production of cholesterol was increased in the presence of cofactors as compared to incubation without cofactors. Fetal liver was found to be capable of synthesizing cholesterol from sodium acetate but incapable of removing the cholesterol side-chain to allow conversion to steroid hormones (Telegdy *et al.*, 1972).

Mori (1976) investigated the incorporation of carbon-14-labeled sodium acetate into hormones by human ovarian follicles. Ovarian follicles were isolated from human ovaries in the follicular and luteal phases; follicles were 5 to 15 mm in size. Minced follicles were incubated with 100 μCi labeled sodium acetate, with or without 100 IU human chorionic gonadotropin (hCG). Dehydroepiandrosterone was the major hormone synthesized from sodium acetate. Minor

products included testosterone, progesterone and pregnenolone. Androstenedione, 17-hydroxyprogesterone, estradiol and estrone were also produced.

The synthesis of progesterone from sodium acetate and cholesterol was measured in the intact ovaries of pregnant rats. On day 16 of gestation, albino Wistar rats were anesthetized and infused for 2 hours via cannula into the parametrial artery with 59.6 mCi/mmol carbon-14-labeled sodium acetate or 58.4 mCi/mmol labeled cholesterol at 0.02 ml/minute. The entire parametrial vein effluent was collected in 15 minute samples through another cannula. Sodium acetate conversion into progesterone was less than 0.5% of the amount of synthesis possible; this figure included the labeled progesterone secreted into the blood throughout infusion as well as the progesterone and precursors pooled in the ovaries when the infusion was complete. Over 50% of the possible progesterone synthesis from labeled cholesterol was found. The authors concluded acetate is of little importance as a blood-borne progesterone precursor in the ovaries of pregnant rats (Swann and Bruce, 1986).

Synthesis of testosterone was studied in decapsulated testes of adult mice. Testes were harvested and then incubated in 500 μCi of 56.2 mCi/mM carbon-14-labeled sodium acetate for 300 minutes. Radio-labeled cholesterol, androstenedione and testosterone were produced. The study was repeated except 10 mIU hCG was added to the media in half the cultures but not the other half. Radio-labeled hormones were measured after 20, 60, 120, 180 or 300 minutes incubation. Androstenedione and testosterone levels were significantly increased and the cholesterol yield was significantly decreased with the addition of hCG. Increasing the hCG by 400-fold (i.e., 40 IU hCG) further decreased cholesterol levels and increased testosterone levels by a factor of four. The addition of 2 μM cyclic-AMP had similar effects. No amounts of pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone or dehydroepiandrosterone, which are supposed intermediates of testosterone synthesis, were produced in any of these incubations. When mouse Leydig cells were incubated in 50 μCi labeled sodium acetate for 180 or 300 minutes, with or without hCG, labeled cholesterol and testosterone were again isolated. Androstenedione was found in small amounts only in the presence of hCG (de la Torre *et al.*, 1976).

Sodium Acetate Toxicity Studies

Sodium acetate ingestion was found to adversely affect thyroid function. Male Long-Evans rats were administered 300 mg sodium acetate/kg diet *ad libitum*; the average daily intake was found to be 21 mg/kg bodyweight daily for 3 months. Bodyweights of treated rats were significantly decreased as compared to unexposed controls. Absolute and relative thyroid weights were significantly increased; relative thyroid weights for treated rats were nearly double the relative weights of controls. Uptake of $^{131}I$ and production of TSH were significantly increased in treated rats. However, $T_3$ production was decreased resulting in a low $T_3$:$T_4$ ratio. When challenged with a single 200 μg iodide load, treated rats had a significantly reduced capacity to bind the iodide (i.e., biotransformation to organic iodine) as compared to iodide challenged controls (Goldman, 1981).

Sodium Acetate Developmental Studies

Sodium acetate was used as the control exposure in a transplacental carcinogenic evaluation of nickel(II) acetate. F344/NCr rats were injected intraperitoneally with 180 μmol sodium acetate/kg bodyweight on day 18 of gestation. At four weeks of age, pups were separated into two groups. One group was exposed to 0.05% sodium barbitol, a known renal tumor promoter, in the drinking water; the other group drank distilled water. Both groups were followed through 85 weeks of age. No gestational abnormalities were reported. There were no deaths in either group until 71 weeks of age; very few deaths occurred prior to the study conclusion. Grade 1 and 2 renal cortical dysplastic foci occurred frequently among males dosed with sodium acetate and sodium barbitol; one incident of grade 3 foci and a single renal cortex adenoma were reported. A low incidence of grade 1 renal cortical dysplastic foci was found among both sets of females and among males exposed to sodium acetate alone. Similarly, a low incidence of pituitary adenomas was seen in both sexes of rats. These lesions were more frequent in female rats and slightly more frequent among males and females exposed to sodium barbitol as well as sodium acetate. No carcinomas were found. The latency period for the pituitary adenomas was 81 or more weeks of age; the latency period of similar lesions in nickel(II) acetate exposed rats was only 58 to 74 weeks of age. Statistical evaluations for sodium acetate exposed rats were not available as they served as the controls for this study (Diwan *et al.*, 1992). Wiebe *et al.* (1982) also used sodium acetate as a control exposure during a gestation and lactation lead acetate exposure study.

Sodium acetate was found to have "antifertility" effects in a multiple species experiment by Dutta and Fernando (1972). Rats were exposed via gavage to 1.0, 5.0, 10.0, 20.0, 100.0, 200.0, 300.0, 400.0 or 500.0 mg/animal on single days of gestation (day 1, 2, 3, 4 or 5 of gestation). Other rats were exposed to daily doses of 1, 5, 10 or 20 mg/day on consecutive days 1 through 5, 1 through 7, 1 through 10 or 6 through 10 of gestation. Control animals were exposed to distilled water gavage; controls had 100% success rate of pregnancy. In general, sodium acetate did result in varying degrees of infertility. Both single doses and consecutive administrations of sodium acetate decreased fertility, although there was no clear evidence of dose-dependency. Examination of the uteri revealed normal physiology and few resorption sites, indicating both pre- and post-implantation losses. Maternal toxicity was not observed. Statistical results were not presented for this study.

Mice were also exposed to sodium acetate via gavage in the 1972 Dutta and Fernando study. Mice were exposed to 1.0 mg/animal daily on gestation days 1 through 7, single gestation days (i.e., 1, 2, 3, 4 or 5), multiple consecutive gestation days (i.e., days 1 and 2, 1 through 3, 1 through 4 or 1 through 5) or days 6 through 10, which is the post-implantation period. A decrease in pregnancy rate was evident for all exposure times. The effect was most pronounced when sodium acetate was administered on two or more consecutive days during the pre-implantation period. Again, all controls had 100% success rate, maternal toxicity was not evident and no statistical evaluations were available for the data.

In the same study, female hamsters were utilized in a similar manner. Hamsters were dosed daily with 10.0, 20.0, 50.0, 100.0, 200.0 or 400.0 mg/animal on days 1 through 10 of gestation. A second set was dosed with 5.0, 10.0, 20.0, 50.0 or 100.0 mg/day on gestation days 1 through 5. Single doses of 200.0 mg were administered to other hamsters on days 1, 2, 3, 4 or 5 of gestation. Hamsters dosed on days 1 through 5 and on days 1 through 10 showed a somewhat dose-dependent decrease in fertility when compared to 100% success in controls. Single

doses were effective at decreasing fertility regardless of the day of administration. Few resorption sites were noted in otherwise normal uteri. No maternal toxicity was observed. Statistical verification of these results were not available (Dutta and Fernando, 1972).

Guinea pigs were also used in this study. Dams were exposed to 5.0, 10.0, 25.0, 50.0, 100.0 or 200.0 mg/day on gestation days 1 through 10. Of the 5 animals in each dose group, a single successful pregnancy was reported in dose groups of 25.0 mg/day or less. Animals receiving 50.0 mg/day or greater did not become pregnant. One dam had an enlarged, but not congested, uterus. Additional maternal toxicity was not evident and statistics were not reported (Dutta and Fernando, 1972).

Finally, rabbits were exposed to 1.0, 5.0, 10.0 or 20.0 mg/day on gestation days 1 through 10 or to 5.0, 10.0 or 20.0 mg/day on days 6 through 10. Fertility was reduced in a variable fashion for all doses administered on days 1 through 10. Of the four dams in each group dosed on days 6 through 10, only one pregnancy was completed. As with the other species, maternal toxicity was not evident, the uteri examined were normal and statistical evaluation was not available (Dutta and Fernando, 1972).

Sodium Acetate Postnatal Studies

Sodium acetate was used as a vehicle control in a behavioral study involving lead acetate exposure. Albino Wistar rat pups were orally exposed to sodium acetate equimolar to 50 mg lead acetate/kg bodyweight on postnatal days 6, 9, 12, 15 and 18. Non-handled control rats were not gavaged and were only weighed periodically. Although no difference was found in neonatal weights of the pups, sodium acetate exposed pups did not gain as much weight during the third week of life (i.e., just prior to weaning) as compared to non-handled controls and lead acetate exposed pups. Bodyweights at the conclusion of the study were not different between groups. All pups were food-deprived in order to start maze pre-training on day 33 of age; maze skills were learned until day 36. Half the pups from each exposure group then underwent sated latent learning of the actual test maze on day 41 and 42. The other pups from each group were evaluated in an open-field (i.e., the actual maze field with no wall), also in a sated condition. Sodium acetate exposed pups were not different from non-handled controls in latent learning; however, open-field activity was significantly increased among the sodium acetate group as compared to controls. On day 45, all pups were food-deprived and were scored in the test maze. As expected, pups that had latent learning made fewer mistakes and traversed the maze more quickly than those with open-field experience. However, sodium acetate pups from either learning group did not differ from non-handled pups with the same learning experience (Massaro *et al.*, 1986). McCarren and Eccles (1983a and 1983b) also used sodium acetate as a control for lead acetate exposure in neonatal behavioral studies.

In another lead acetate behavioral effects study, newly weaned rats were exposed to sodium acetate as a negative control. Male Long-Evans hooded rats were exposed to 50 or 500 ppm sodium acetate in drinking water for 34 days prior to behavioral testing starting on day 55 of age. Exposure was maintained throughout testing. Fixed ratio reinforcement behavioral testing was performed with the reinforcement ratio increasing from 1 to 100 (i.e., a food pellet reinforcement for every 1, 5, 10, 25, 50 or 100 lever presses) after a set number of sessions at each ratio step. Patterns of performance have been noted for different reinforcement schedules; these patterns are general across the species and do not fluctuate greatly between

normal rats of different strains. Sodium acetate treated rats served as the controls in this study; abnormal performance patterns were not noted (Cory-Slechta, 1986).

Sodium Acetate *In Vitro*/Screening Studies

During the examination of the growth and production of hCG by cells from a human ovarian papillary cystadenocarcinoma (cell line 163), the effect of sodium acetate and sodium butyrate on these cells was evaluated. Cell line 163 was incubated with 0.2, 1.0, 4.0 or 10.0 mM sodium acetate for 11 days. Sodium acetate did not affect the growth rate or hCG production of cell line 163 as compared to pure media cultured controls. hCG titer in the urine or serum generally reflects tumor burden. hCG production is most commonly associated with ovarian epithelial cancers, although it has been reported in patients with benign disorders and in healthy patients (Kanabus *et al.*, 1978).

Sodium acetate was used in a validation study of an improved cultured rat embryo assay. Rat embryos at 9 days of gestational age were cultured for 2 days in rat serum or human serum with 3% rat serum plus 1 mg glucose/ml. The embryos were then injected with 100 ng sodium acetate per embryo directly into the vitelline circulation of the embryo yolk sac. Direct injection into the circulatory system of the fetus bypasses the metabolically active yolk sac through which the chemical would have to pass during the standard rat embryo assay prior to encountering developing fetal tissues. The embryos were cultured for an additional 24 hours prior to scoring. A numerical score was calculated using these parameters: yolk sac diameter, crown-rump length, somite number, yolk sac protein and DNA, embryo protein and DNA, fore-and hind limb bud development, neural tube integrity, heart beat, yolk sac and embryo blood patches and other abnormalities. Sodium acetate, previously identified as non-teratogenic in animal studies, scored between 27.5 and 30. Teratogens had scores lower than 25; non-teratogens had significantly higher scores than teratogens (Cumberland *et al.*, 1994a). A previous report of these results indicated that sodium acetate caused only yolk sac and embryo blood patches (Cumberland *et al.*, 1994b).

In a study of the effects of lead acetate on steroid hormone production of Leydig cells, sodium acetate effects on hormone production were also compared to levels of production in unexposed control cells. Leydig cells were harvested from 90 day old male Sprague-Dawley rats. These cells were cultured for 48 hours with 50 mIU/ml hCG or hCG plus 200, 500 or 1000 μM sodium acetate. There was no difference between the levels of testosterone and progesterone produced in sodium acetate/hCG treated or hCG treated control cells. Cells treated with hCG plus 100, 250 or 500 μM lead acetate produced significantly less of both hormones in a dose-dependent manner. Sodium acetate was used as a control dose for other lead acetate *in vivo* and *in vitro* assays by these authors (Thoreux-Manlay *et al.*, 1995).

While assessing the effects of 2-methoxyacetic acid on cultured mouse embryos, Stedman and Welsch (1989) evaluated the attenuative effects of sodium formate or acetate. CD-1 mice embryos were harvested on gestation day 11 and exposed to 5 mM sodium acetate for 5 hours. [$^3$H]Thymidine was added and the embryos were cultured for an additional hour; the rate of DNA synthesis was measured by the uptake of this labeled amino acid. The amount of DNA within exposed embryos was not different from control embryos incubated in pure media and [$^3$H]thymidine. The rate of DNA synthesis in acetate exposed embryos was also not statistically different. Exposure to 24 mM 2-methoxyacetic acid, the teratogenic metabolite of 2-

methoxyethanol, resulted in a 50% reduction in DNA synthesis in the treated embryos. Embryos incubated with 2-methoxyacetic acid and sodium acetate had normal levels of DNA synthesis. Sodium acetate was found to attenuate teratogenicity of 2-methoxyacetic acid at five times the effective concentration of sodium formate (i.e., 1 mM sodium formate had the same effect as 5 mM sodium acetate).

In an evaluation of the mouse stem-cell assay, sodium acetate was found non-teratogenic. The D-3 embryonic stem cell (ESC) line, routinely used in the manufacture of transgenic mice, was maintained in an undifferentiated state with leukemia inhibitory factor (LIF). Without LIF, the ESC differentiated into embryonic endoderm cells which are morphologically different from the original stem cells. The ESC were able to be harvested daily, without the use of animals beyond the original donors. After removal from the LIF culture medium, known quantities of ESC cells were transferred to 96 well plates and allowed 2 hours to attach to the well. The cells were then exposed to the test substance at varying concentrations and incubated for seven days. ESC cells were exposed to sodium acetate at concentrations up to 500 μg/ml. A teratogen would have inhibited ($IC_{50}$) cell differentiation (D) at less than half the concentration at which cytotoxicity (C) occurred (i.e., C/D > 2). Sodium acetate did not cause cytotoxicity or inhibit differentiation at the levels tested, so the C/D ratio was effectively 1 (i.e., >500 μg/ml / >500 μg/ml) (Newall and Beedles, 1994).

In an investigation of cholinomimetic compounds, chemicals that act similarly to acetylcholine on cholinergic nerve receptors, sodium and potassium acetate were assessed for their ability to reduce teratogenicity of these compounds. White Leghorn eggs were injected with 1.5 mg carbachol/egg, carbachol plus 10 mg sodium acetate/egg or sodium acetate alone. Injections were made into the yolk sac on the fourth day of incubation. Embryos were examined on day 19 of incubation. Embryos exposed to sodium acetate alone gave no indication of interference with normal chick development. Sodium acetate administered with carbachol did not affect the level of teratogenicity caused by carbachol alone. Cholinomimetic compounds can cause abnormal cervical vertebrae development resulting in a short, crooked neck, beak deformities and abnormal leg musculature (Landauer, 1975).

Verrett *et al.* (1980) evaluated the effects of food additives, including sodium acetate, on the developing chick embryo. Fertile Single-Comb White Leghorn chicken eggs were injected with up to 10 mg sodium acetate/egg either prior to incubation or on the fourth day of incubation. Injections were made into the yolk or into the air sack. Sodium acetate showed no evidence of teratogenicity. The $LD_{50}$ was estimated to be 4.58 mg/egg in an intra-yolk sac injection prior to incubation. Toxicity at 4 days of incubation was found to be higher among all 80 chemicals tested than the toxicity in preincubation eggs. The authors attempted to use concentrations consistent with human food exposure levels.

Sodium acetate was used to evaluate a primary cell screening assay using chick embryo neural retina cells (CERC). White Leghorn chicks were incubated for 6.5 days prior to the neural retina cells being harvested. The cells were then dissociated into single cell units and incubated with the test chemical in a standard medium for 24 hours. Sodium acetate was tested in concentrations up to 40 mM. After 24 hours exposure, the cells were cultured in medium alone for an additional 6 days in standard media before assessment. Dissociated cells kept in a rotating suspension form aggregates in the first 24 hours; aggregates tend to be of similar size regardless of the initial cell density. The original neural cell layers recreate within

the aggregates and differentiate over time. A teratogen would affect aggregate size, growth, as measured by total protein content of the aggregate, and differentiation, as measured by glutamine synthetase content (Daston *et al.*, 1991). Glutamine synthetase protein indicates phenotype differentiation as it is abundantly present in mature retinas but is only expressed under hormonal stimulation, which can be suppressed by inhibitors of gene expression (Daston *et al.*, 1995). Sodium acetate did not affect size, growth or differentiation of the CERC at these concentrations (Daston *et al.*, 1991).

In 1995, Daston *et al.* replicated the previous study with more chemicals in a blind manner so the researchers did not know the chemical identities during the study. Sodium acetate was tested in concentrations up to 61 mM. At 61 mM sodium acetate, aggregate size was significantly affected as compared to control CERC. Growth and differentiation were not affected, establishing a lowest observed effect concentration (LOEC) of 61 mM. The authors compared their results with *in vivo* rat lowest observed effect levels (LOELs) found in literature; sodium acetate had a LOEL of >194.2 mmol/kg based on an intravenous rat study in which the highest dose did not cause adverse effects (Miller, 1971). Therefore, a true LOEC/LOEL ratio was not calculable. The authors listed sodium acetate as a nondevelopmental toxicant for CERC (Daston *et al.*, 1995).

## Potassium Acetate

Potassium acetate is a liquid and can be used as a deicer or as a pre-wetting agent. Potassium acetate melts snow and ice faster than the glycols, has a longer residual effect and is less slippery. Although potassium acetate's BOD is lower than urea or the glycols, it breaks down at lower temperatures, resulting in a higher BOD under very cold conditions as compared to the glycols or urea. Potassium acetate works most effectively if applied before ice forms, preventing the ice to pavement bond. Potassium acetate is somewhat cheaper to use than the glycols (Mericas and Wagoner, 1996).

Only two sodium formate studies were located in this literature review; no *in vivo* studies were found. Two screening assays using chick embryos and fruit fly embryonic cells did not indicate high teratogenicity potential. Further research would be necessary before the endocrine activity of sodium formate could be determined.

### Potassium Acetate *In Vitro*/Screening Studies

In an investigation of cholinomimetic compounds, chemicals that act similarly to acetylcholine on cholinergic nerve receptors, sodium and potassium acetate were assessed for their ability to reduce teratogenicity of these compounds. White Leghorn eggs were injected with 1.5 mg carbachol/egg, 1.5 mg carbachol plus 7.5 mg potassium acetate/egg, 2.0 mg carbachol/egg, or 2.0 mg carbachol plus 5 mg potassium acetate/egg. Additional eggs were injected with 3.5 mg tetramethylammonium chloride (TMA)/egg, 3.5 mg TMA plus 7.5 mg potassium acetate/egg, 10 mg TMA/egg or 10 mg TMA plus 7.5 mg potassium acetate/egg. Control eggs were injected with up to 20 mg potassium acetate/egg. Injections were made into the yolk sac on the fourth day of incubation; embryos were examined on day 19 of incubation. Embryos exposed to potassium acetate alone gave no indication of interference with normal development. Potassium acetate administered with carbachol or TMA significantly decreased the level of

teratogenicity caused by carbachol or TMA alone. Cholinomimetic compounds can cause abnormal cervical vertebrae development resulting in a short crooked neck, beak deformities and abnormal leg musculature. Co-administration of potassium acetate decreased the incidence of short/crooked necks and leg muscle hyperplasia in the low dose group of each cholinomimetic compound. Similarly, potassium acetate was only able to decrease the leg deformities in the high dose group of either teratogen (Landauer, 1975).

In a 100 chemical verification of the *Drosophila* embryonic cell culture teratogenic assay, potassium acetate was not found to have a high teratogenic potential. *Drosophila* eggs from 3 wild strains were collected within 3.5 hours of oviposition (i.e., prior to overt structural differentiation), dechorionated and sterilized. The eggs were homogenized and the embryonic cells were plated into cell culture dishes with standard medium. After allowing 15 to 20 minutes for the cells to adhere to the dishes, the culture medium was replaced with the test chemical in medium or fresh medium for controls. After 24 hours, ganglia and myotube differentiation was evaluated. A chemical would be classified as having a high potential for teratogenicity if a statistically significant decrease in muscle and nerve tissue differentiation is observed. Each test substance was evaluated in three separate trials. Potassium acetate was tested at $10^{-3}$ M. Ganglia and myotube differentiation was 78 and 73%, respectively, of the differentiation observed in parallel control cultures grown on the same day; these reductions were not statistically significant (Bournias-Vardiabasis *et al.*, 1983).

## Calcium Magnesium Acetate

Although calcium magnesium acetate (CMA) has been used for years as a highway deicer where corrosion of concrete is a concern (i.e., on concrete spallings and bridges), it is not widely used on runways as it is slower acting than other deicers. Additionally, CMA costs about 1.5 times the cost of urea to use and CMA has a higher BOD than sodium acetate or formate (Mericas and Wagoner, 1996).

Little was found about calcium magnesium acetate, commercially known as Ortho Ice-B-Gon™, except that it is composed primarily (91%) of calcium acetate, magnesium acetate and corrosion inhibitors. Chevron, its manufacturer, has performed the necessary acute toxicity tests (CEHC, 1991). Outside of the subchronic toxicity test described below, no other toxicity information was available on this chemical. The endocrine activity of CMA is impossible to predict from the following study alone.

### Calcium Magnesium Acetate Toxicity Studies

In a subchronic oral toxicity test, male and female rats were orally dosed with calcium magnesium acetate (Ortho Ice-B-Gon™). Doses of 0.10, 0.33 or 1.00 g/kg bodyweight were given daily, 5 days per week, for 4 weeks. The high dose would correlate to approximately 2.5 ounces calcium magnesium acetate per day for a 70 kg human. Mortality did not occur at any dose level. Bodyweights, food consumption and organ weights were not different from controls given 1.0% (w/w) carboxymethylcellulose in water. Slight effects were limited to a red nasal discharge found primarily among males in the mid and high dose groups and an increase in mean cell hemoglobin among high dose females and males at all dose levels. The authors

reported no significant effects for calcium magnesium acetate up to 1.00 g/kg. Statistical evaluations were not discussed (CEHC, 1987).

## Acetic Acid

Acetic acid is an expected breakdown product of sodium, potassium or calcium magnesium acetate salts in water. Few studies were found for acetic acid in this review. Those available point to potential endocrine activity, similar to sodium acetate. Acetic acid can act as a metabolic precursor of triacylglycerols and phospholipids. Acetic acid was used as a control compound for a lead acetate lactation exposure; physical and developmental effects resulted from acetic acid. Two *in vitro* studies showed no effect on insulin secretion but altered pancreatic cell reactivity resulting in decreased insulin levels. Further testing and mechanism determination would be indicated to clarify the effects of sodium acetate on the endocrine system.

### Acetic Acid Kinetics Studies

In 1984, del Hoyo *et al.* examined the androgenic control of phospholipid and triacylglycerol synthesis from acetate. The ventral prostates from young male Wistar rats were harvested. After pre-incubation for 30 minutes, the prostates were incubated with 2 mCi carbon-14-labeled acetate for 2 hours. The acetate was mainly incorporated into phospholipids; radio-labeled levels of free fatty acids and triacylglycerols were low. Additional ventral prostates were incubated in testosterone or dihydrotestosterone, its main active metabolite, for two hours prior to acetate exposure. The presence of androgens increased the amount of labeled acetate incorporated into phospholipids and decreased triacylglycerol levels further. Testosterone had greater effects than dihydrotestosterone. These results were confirmed *in vivo*. Rats were castrated and monitored for seven days; androgens were not detectable in plasma after this time. Testosterone or dihydrotestosterone was injected subcutaneously and labeled sodium acetate was injected into each lobe of the ventral prostate. Androgens again decreased acetate incorporation into triacylglycerol and increased phospholipid production. Again, greater effects were obtained with testosterone.

### Acetic Acid Lactation Studies

Barrett and Livesey (1982) investigated the effects of acetic acid when used as a control in lead acetate studies. The authors cited an unpublished pilot study in which sodium acetate was used as a control for lead acetate exposure of rat dams through lactation. Both sodium acetate and lead acetate exposed pups had similar mortality rates and both groups of dams tended to consume less food and water than distilled water controls. Recognizing that the optimum sodium intake was likely being exceeded when using sodium acetate as a control, acetic acid was tested as a control exposure for lead acetate studies. Albino Wistar rats nursing 8 male pups were exposed via drinking water to $5.2 \times 10^{-6}$ M acetate ions (i.e., 0.3 ml glacial acetic acid/l water), $2.6 \times 10^{-6}$ M lead acetate (i.e., $5.2 \times 10^{-6}$ M acetate ions) or distilled water. Dams were exposed throughout lactation from day 1 after parturition through day 18, when pups consume quantities of water and food on their own. No significant difference was seen in food and water intake of the dams. However, acetic acid exposed pups had significantly higher

bodyweights as compared to either the lead acetate or control pups at eight days of age. Acetic acid pups continued to be significantly heavier than water controls until 20 days of age. Lead acetate pups were heavier than controls but lighter than acetic acid pups. Post-weaning weights of any group of pups was not different from the others, indicating that discontinuing acetate ion exposure resolved the weight difference. All pups were tested for open field activity on days 20, 28 and 44 of age. Acetic acid pups were found to have significantly decreased activity as compared to lead acetate or control pups on day 44. Pups exposed to lead acetate displayed activity levels higher than acetic acid pups but lower than distilled water controls. Acetic acid is capable of producing effects in rats exposed during lactation; the authors indicate that the use of metallic lead in behavioral experiments would overcome the need to control for acetate ion effects.

Acetic Acid *In Vitro*/Screening Studies

Tiengo *et al.* (1981) explored the effects of ethanol and its known metabolites, acetaldehyde and acetate, on the rate of insulin and glucagon secretion in isolated perfused rat pancreases. Sprague-Dawley rat pancreases were isolated, equilibrated for 20 minutes under standard perfusion conditions and infused for 20 minutes with 1 mM acetate. The functional integrity of the pancreas was then measured by rapid infusion of 11.1 mM glucose followed by 30 more minutes of perfusion. Alternatively, pancreases were infused with acetate for 20 minutes followed by 20 mM arginine infused and another 20 minutes perfusion. Insulin and glucagon secretion were not affected in the first 20 minutes of perfusion, prior to the infusion of glucose or arginine. Acetate was found to significantly inhibit insulin secretion after the 11.1 mM glucose infusion; this inhibition was evident in the first five minutes of perfusion as well as the second stage (i.e., 5 to 30 minutes). Insulin secretion was also significantly inhibited after arginine administration; this effect was most pronounced in the first five minutes of the following perfusion. Conversely, acetate perfusion followed by arginine infusion resulted in increased glucagon secretion; the increase was significant throughout both stages of the following perfusion (i.e., all 20 minutes). Acetate did not affect the baseline secretion of insulin. Therefore acetate has no primary effect on pancreatic $\alpha$- and $\beta$-cell secretion; however, acetate did alter the cells' reactivity to secretory stimuli.

Similarly, Potter *et al.* (1982) investigated the effects of ethanol and its metabolites on the immunoreactive insulin release from perfused rat pancreatic islets. Pancreatic islets were isolated from adult male Sprague-Dawley rats and allowed to equilibrate in the perfusion chamber. The islets were first perfused with 30 mg/dl glucose (basal level) and 0.29, 2.9 or 29 mg/dl acetate for 30 minutes. A stimulatory concentration of glucose (300 mg/dl) plus acetate was then perfused for another 30 minutes. Finally, a 20 minute recovery period with only basal levels of glucose was performed. Acetate, in concentrations up to 29 mg/dl, did not significantly affect the basal output or the stimulated output of immunoreactive insulin. Acetaldehyde, the primary metabolite of ethanol, inhibited immunoreactive insulin release only at high levels not achievable *in vivo*. Ethanol was found to prevent insulin release in a dose-dependent manner, confirming that this effect is due to the parent compound and not the metabolites.

## JET FUELS AND RELATED HYDROCARBONS

Petroleum hydrocarbon remediation is a serious concern for the U.S. Air Force. In 1994, over 4400 Air Force sites had been identified for some level of cleanup; fuel contamination was estimated to be the contaminant of concern for 50 to 60% of these sites. Risk-based approaches to remediation include determination of BTEX (benzene, toluene, ethylbenzene and xylenes) concentrations remaining at the site (Miller, 1994). Aromatics such as BTEX are fairly well known toxicants and therefore have a high priority of concern, even though, for example, the jet fuel JP-8 contains only approximately 18% aromatics by volume. These aromatics include benzene, alkyl benzenes, toluene, xylene and others (COT, 1996). More volatile fuels such as JP-4 would contain more aromatics while heavier fuels such as diesel would contain less. Because jet fuel and other petroleum contaminants are a cleanup and an occupational concern in the Air Force, this literature review contains available information on the endocrine activity of not only jet fuels but also diesel and toluene, ethylbenzene and xylene (i.e., the less well studied members of BTEX).

### Jet Fuels

As relatively few jet fuel studies exist, different jet fuel formulations were assessed together. Jet fuels do not appear to target organs associated with the endocrine system. The formulations developmentally tested *in vivo* were found non-teratogenic. Chick and duck embryo assays were also negative. As exposure to older jet fuel formulations occurs from environmental sources and exposure to current formulations occurs in occupational settings, performance of an endocrine test battery on individual formulations would be justified.

#### Jet Fuel Toxicity Studies

The U.S. Air Force has completed the transition from JP-4 to JP-8 (Mattie *et al.*, 1996), which is similar to commercial Jet Fuel A (Mattie *et al.*, 1991). Male Sprague-Dawley rats gavaged daily with neat JP-8 for 90 days had significant dose dependent decreases in bodyweight as compared with controls given distilled water. JP-8 was dosed at 750, 1500 or 3000 mg/kg; the rats were sacrificed approximately four hours post-exposure. Hydrocarbon ($\alpha$ 2-microglobin) nephropathy, a male rat specific effect, accompanied the decrease in bodyweight gain. Although the relative testicular weight was significantly increased in the high dose group, this appears to be related to the severe depression in the bodyweights of these animals; absolute testicular weights were not different from controls (Mattie *et al.*, 1995).

Prior to JP-8, the Air Force used JP-4 jet fuel. Utilizing a 12-month intermittent exposure, the oncologic potential of JP-4 was determined in rats and mice. Fischer 344 rats were exposed to 1000 or 5000 mg/m$^3$ JP-4 for 6 hours/day, 5 days per week except holidays, for a year. Post-exposure, 90% of the animals were held for a 12-month observation period. Pulmonary neoplasms were not significantly increased. Treated male rats displayed hydrocarbon nephropathy and the associated renal toxicity, decreased weight gain and neoplasia. Female rats did not display significant treatment related effects. Further pathological findings were considered equivocal or the product of species variation. These findings included a significant increase in prostate cystic degeneration, testicular interstitial cell tumors, female mammary cystic hyperplasia and male mammary fibroadenomas among the high exposure group during

the observation period or immediately following exposure. Also noted were increased pituitary adenomas and carcinomas in the male low exposure and the female high exposure groups (Bruner *et al.*, 1993).

C57BL/6 mice were used in the same study and exposed under the same conditions. Again, pulmonary neoplasms were not significantly increased. Females showed a significant increase in benign hepatocellular adenomas; however, males showed a significant decrease of the same. Further pathological findings were considered as equivocal or the product of species variation. These findings included significantly decreased pituitary carcinomas in the female low exposure group and significantly increased seminal vesicle dilation in male high exposure animals. Increased testicular atrophy and interstitial cell hyperplasia were noted at both exposure levels and the high exposure level, respectively. Testicular lesions were associated with chronic skin disease caused by dominance fighting (Bruner *et al.*, 1993).

Shale and petroleum derived Navy jet fuel, JP-5, was tested in Fischer 344 rats during an inhalation toxicology study. Rats were exposed to 150 or 750 mg/m$^3$ JP-5 continuously for the 90-day exposure period and then sacrificed at 0, 19 and 21 months post-exposure. Hydrocarbon nephropathy, including the accompanying increased kidney weights, increased serum creatinine and BUN levels and decreased bodyweight gains, occurred in a dose dependent manner in male rats exposed to either JP-5 formulation. Female rats displayed no renal damage and suffered only hepatocellular vacuolization and fatty changes when exposed to shale derived JP-5. Male rats showed a significant increase in pituitary adenomas and thyroid hyperplasia when exposed to petroleum low and high doses, respectively. Shale JP-5 led to increased thyroid c-cell and adrenal pheochromocytomas (i.e., usually benign adrenal medullary tumor that secrets catecholamines) in male rats again at the low and high doses, respectively. Female rats displayed significantly increased incidence of mammary gland hyperplasia at both the petroleum low and high doses and at the shale low dose. Thyroid hyperplasia was increased at the petroleum high dose while pituitary adenomas were increased in the shale low exposure group. Not only were these results not dose dependent, but endocrine lesions, especially pituitary tumors, are reported to be extremely common among aging Fischer rats. Additionally, thyroid tumors may be a secondary effect of hydrocarbon nephropathy due to extended imbalance of Ca:$PO_4$ levels. Shale and petroleum derived JP-5 were not found to differ significantly in toxicity (Gaworski *et al.*, 1985).

C57BL/6 mice were also used in this JP-5 study under the same exposure conditions. Hepatocellular vacuolization and fatty changes in the liver were the major effects reported. A significant increase in endometrial (uterine) cysts was observed among the petroleum high dose group; endometrial cysts were significantly decreased among shale high dose animals. Pituitary carcinomas were also significantly decreased in both the low and high shale exposure groups. Both types of tumors were frequently found among all groups, including controls, as they are common among aging mice. Again, shale and petroleum derived JP-5 were not found to differ significantly in toxicity. Beagle dogs were also studied; endocrine specific organ weights were not reported in this species (Gaworski *et al.*, 1985).

Jet Fuel Developmental Studies

In the International Agency for Research on Cancer (IARC) review of carcinogenic risks to humans (1989a), a rat inhalation study with Jet Fuel A was described. Charles River CD dams

were exposed to 100 or 400 ppm Jet Fuel A for 6 hours daily on gestation days 6 through 15. Embryotoxic, fetotoxic or teratogenic effects were not observed (Beliles and Mecler, 1982). In a different chapter of the same review (1989b), a similar study exposed rats to kerosene of unspecified composition (i.e., the kerosene could be aviation grade or fuel oil grade). Dams were again exposed to 100 or 365 ppm kerosene 6 hours daily on days 6 through 15. Similarly, no teratogenic effects were seen (Schreiner, 1984).

Jet Fuel A and JP-8 are both kerosene type aviation fuels (Mattie *et al.*, 1991). In a review of the teratogenicity of solvents, Schardein (1993) uncovered a kerosene teratogenicity study. Kerosene was found non-teratogenic in rats, according to 1979 unpublished data obtained under the Freedom Of Information Act (FOIA).

JP-10, the major component used in cruise missile fuel, is a synthetic saturated polycyclic hydrocarbon. JP-10 tested in pregnant Fischer 344 rats did not prove teratogenic but was embryotoxic at maternally toxic levels. Rats were exposed by inhalation to 600 ppm or by gavage to 250, 500 or 1000 mg/kg-day JP-10 over days 6 through 15 of gestation. Dams and pups were examined on day 20. No significant signs of toxicity were noted at the 250 mg/kg-day level. The 500 mg/kg dams showed significantly decreased weight gain during the first part of exposure (days 6 through 10 of gestation). The 1000 mg/kg-day and 600 ppm dose levels had similar effects. Both caused significant decreases in weight gain over the entire period of exposure; tremors were noted in many animals and a few had minor convulsions. Only the 1000 mg/kg group, however, demonstrated decreased bodyweights over both the exposure and recovery period combined (days 6 through 20). Incidence of malformation was not increased over pure air inhalation or corn oil gavage controls. Embryotoxicity was limited to a significant decrease in fetal weight in the 500 mg/kg-day exposure group that was not present in the 1000 mg/kg-day or the 600 ppm animals; significant resorption of greater than 25 percent of the litter occurred in the 1000 mg/kg-day exposed animals but not in the inhalation dose group. In a concurrent toxicokinetic study, rats were exposed to 600 ppm on day 19 of gestation. Groups of rats were removed from exposure at 15, 30, 60, 120 and 240 minutes. Maternal blood levels of JP-10 leveled out at one hour; fetal blood levels required longer to reach a plateau of roughly one-half the maternal level (Keller *et al.*, 1983).

Lyng (1981) found no evidence of teratogenicity or fetotoxicity in mice exposed to JP-10. Pregnant ICR mice were orally dosed with 0.2, 0.4, 0.6 or 0.8 ml/kg daily on days 6 through 9 of gestation. JP-10 did not affect the number of implantations or resorptions and viability of fetuses. Soft tissue and skeletal anomalies were not increased as compared to controls. The only significant difference was an increase in weight of the 0.2 and 0.4 ml/kg-day exposed fetuses as compared to controls; 0.6 and 0.8 ml/kg-day group weights were not different from control group weights.

Jet Fuel *In Vitro*/Screening Studies

Aviation kerosene (commercial Jet Fuel A) was evaluated in a mallard duck egg assay. Three to six dose volumes of neat fuel were streaked on the eggs below the air space, avoiding blockage of pores which would prevent necessary oxygen uptake. The dosing took place on day 3 of development (third day after incubation began); day 3 is critical to organogenesis and is comparable to day 2 in chicken embryos. The $LD_{50}$ for Jet Fuel A in mallard eggs was greater than 50 µl/egg, as no mortality was seen at the doses tested. Due to insufficient

evidence of external malformations, soft tissue and skeletal examinations were not performed on day 18 when the embryos were evaluated. The low toxicity of Jet Fuel A as compared to other petroleum cuts tested was attributed to its relatively low aromatic hydrocarbons content (Hoffman and Albers, 1984).

In an earlier study, Albers and Gay (1982) tested weathered or unweathered aviation kerosene on the shells of duck eggs. Either form was applied to the shell in doses ranging from 1 to 20 µl/egg on day 6 of incubation. No toxic effects on the duck embryos were found (IARC, 1989b).

## Diesel

Similarly, few diesel studies were located in this literature review; however, some evidence of endocrine activity was found. Diesel caused thyroid changes in treated rats but was reported non-teratogenic in another rat study. The single *in vitro* developmental test found was an ecological teratogenicity test which reported malformations in frog embryos. Further endocrine testing would be warranted.

### Diesel Toxicity Studies

In a rat dermal toxicity study, high-boiling point coal liquefaction product was compared to diesel fuel. Male and female Sprague-Dawley rats were shaved in the interscapular region and dosed with 400 mg/kg neat diesel daily, 7 days a week for 6 weeks. Examination following exposure revealed a significant decrease in bodyweight gain among the males for all six weeks of treatment and among females at the second week of treatment as compared to controls. However, food consumption among exposed animals compared to control animals was decreased only in the second and third weeks for the males and in the third through sixth weeks for the females. Microscopic changes were noted in thyroids of treated animals; changes included decreased colloid density, collapsed thyroid follicles and epithelial height increases. Mild cytoplasmic vacuolations were also noted. Although some animals had enlarged adrenal glands and lymph nodes, histological abnormalities were not noted (Chu *et al.*, 1988).

### Diesel Developmental Studies

In the IARC review of carcinogenic risks to humans (1989c), a rat inhalation study using diesel fuel of unspecified grade was reported. Dams were exposed to 100 or 400 ppm diesel for 6 hours daily on days 6 through 15 of gestation. No teratogenic effects were seen (Schreiner, 1984).

### Diesel *In Vitro*/Screening Studies

FETAX (Frog Embryo Teratogenesis Assay - *Xenopus*) testing performed on extracts from gasoline and diesel contaminated soils showed embryo lethality and teratogenicity. Extracts of moderately (1100 - 7 mg/kg gasoline and 160 - 350 mg/kg diesel) and highly (10000 - 430 mg/kg gasoline and 600 - 18000 mg/kg diesel) contaminated soils were tested. Percent malformation was significantly increased over control levels in both moderate samples. Percent

mortality as well as malformation rates were significantly higher in assays of both highly contaminated extracts. Malformations observed included gut miscoiling, craniofacial defects and microophthalmia, microencephaly and hemorrhage (Fort *et al.*, 1995).

## Toluene

Numerous toluene studies were found through this literature review. The majority indicate that toluene is capable of endocrine activity. Toluene was found to cause thyroid effects, change brain catecholamine and hormone levels and affect adult and neonatal behavior in rodents. Although there was little evidence of male reproductive toxicity *in vivo*, *in vitro* tests found sperm and fertilization adverse effects. Most developmental studies reported toluene as a fetotoxic but not teratogenic compound; embryotoxicity was also found *in vitro*. Gestational exposure to toluene also resulted in behavioral changes of the offspring. Human occupational/epidemiological studies appear to substantiate laboratory studies. Occupational studies of male printers positively correlated toluene exposure with serum hormone changes. Although toluene exposure was not found to affect menstrual cycles in the workplace, significant fetal effects including teratogenicity were found in babies exposed *in utero* to the extremely high levels of toluene from paint sniffing. Further studies to demonstrate the mechanism of fetotoxicity would be of assistance in proving endocrine disruption.

### Toluene Toxicity Studies

An inhalation toxicity study of toluene, as well as methanol and methanol/toluene mixtures, demonstrated thyroid effects from toluene exposure. Male and female Sprague-Dawley rats were exposed to toluene at 30 or 300 ppm for 6 hours per day, 5 days per week for 4 weeks. Mild to moderate reduction in thyroid gland follicle size was observed at significant levels in females only; incidence and severity of follicle size reductions were higher among low dose females than high dose females. No clinical signs of toxicity were observed. Other toluene effects included increased serum alkaline phosphatase activities in high dose group males and mild nasal passage histopathological changes in exposed males and females. Significantly increased relative liver and heart weights were observed among low dose males but not among females or high dose animals (Poon *et al.*, 1994).

### Toluene Neuroendocrine Studies

Toluene was shown to affect catecholamine and hormone levels in a subacute exposure study performed by Andersson *et al.* (1980). Male Sprague-Dawley rats were exposed to 500 ppm toluene for 6 hours per day on 3 consecutive days; the rats were sacrificed 16 to 18 hours post-exposure. A significant increase in catecholamine levels, indicated by catecholamine fluorescence, was seen in the lateral palisade zone of the median eminence; this area contains mainly dopamine receptors. Levels in other median eminence and thyroid areas were not different from controls. Corticosterone levels were significantly increased as compared to controls. In a subsequent study, male rats were exposed to 1000 ppm toluene for 6 hours per day for 4 consecutive days and 4 hours on the fifth day prior to sacrifice. Alternatively, a group of rats was given $\alpha$-methyl-tyrosine methylester, a tyrosine hydroxylase inhibitor to indicate catecholamine turnover, followed by 2 more hours of toluene exposure at 1000 ppm. Exposed

rats without inhibitor showed a significant increase in catecholamine levels within the subependymal layer of the median eminence; noradrenaline fluorescence would predominate in this area. Prolactin levels were significantly increased as compared to controls. Exposed rats given the inhibitor showed increased catecholamine turnover rates in the subependymal and medial palisade layers of the median eminence as well as several areas of the hypothalamus where noradrenaline receptors predominate. FSH secretion was significantly increased in these rats. Overall, toluene was found to increase dopamine and noradrenaline stores in the median eminence and also increase noradrenaline turnover in the median eminence and hypothalamus. Changes in hormone levels also resulted. Although this exposure exceeded the threshold limit value (TLV), the higher elimination rate of rats versus man and the higher uptake of solvents during physical work should be considered.

A later study by Andersson *et al.* (1983) confirmed these neuroendocrine effects. Male Sprague-Dawley rats were exposed to 80, 500, 1500 or 3000 ppm analytical grade toluene (i.e., impurity of 14 ppm benzene) for 6 hours per day over 3 consecutive days. A portion of each exposure group was intraperitoneally injected with the tyrosine hydroxylase inhibitor α-methyl-tyrosine methylester two hours prior to sacrifice. All rats were sacrificed within 16 to 18 hours post-exposure. Exposure these toluene concentrations significantly increased noradrenaline levels in several areas of the hypothalamus plus dopamine and noradrenaline levels in each of the examined areas of the median eminence. Only prolactin secretion was significantly increased with exposure as compared to air-exposed controls. Exposure to toluene plus the inhibitor resulted in increased noradrenaline turnover rates in several areas of the hypothalamus and increased dopamine and noradrenaline rates in two of three areas of the median eminence. Hormone secretion levels (i.e., FSH, luteinizing hormone, prolactin, corticosterone, growth hormone and TSH) were not different in toluene and inhibitor exposed rats as compared to air and inhibitor exposed controls. Noradrenaline nerve terminals in the hypothalamus control thermoregulation, cardiovascular regulation and food/water intake; this study supports possible toluene interference with these functions at some exposure level. Unfortunately, statistical significance data for each exposure level was not available.

The effect of toluene exposure on dopamine $D_2$ agonist binding was examined by Hillefors-Berglund *et al.* (1995). Male Sprague-Dawley rats were exposed to toluene (99% pure) at 40, 80, 160 or 320 ppm for 6 hours, 5 days per week for 4 weeks. The rats were observed for 29 to 40 days post-exposure and then sacrificed. Although body, whole brain and caudate-putamen weights were not significantly affected, wet weights of the subcortical limbic area were significantly decreased starting at 80 ppm. [$^3$H]Raclopride was used to test $D_2$ agonist binding affinity. The inhibition constant and the inhibition constant for low-affinity sites of dopamine on [$^3$H]raclopride-binding in the caudate-putamen region were significantly decreased as compared to air exposed control binding affinity; the inhibition constant for high-affinity sites and the proportion of high-affinity sites were also decreased in a dose dependent but not significant manner. These parameters were not significantly affected in the cortical limbic region. Serum prolactin levels were not different from controls at 29 to 40 days post-exposure. The Swedish TLV for toluene is 50 ppm; at 80 ppm and above, toluene was found to lead to persistent increases in affinity of dopamine $D_2$ agonist binding with no effect on the number of $D_2$ receptors. This effect could have behavioral consequences as increased $D_2$ agonist binding affinity has been related to enhanced apomorphine-induced locomotor activity.

Toluene exposure was found to decrease sleeping time, increase activity and water consumption and disrupt neurochemicals in Sprague-Dawley rats. Male rats implanted with

electroencephalogram (EEG) and electromyogram (EMG) electrodes were exposed intraperitoneally to 100 or 200 mg toluene/kg bodyweight daily for 14 days. Sleep patterns were monitored on four pre-exposure days, the last day of exposure and four post-exposure days. Rats in the high exposure group displayed a significant decrease in total sleep time during the light period on the first day following exposure as compared to their sleep patterns prior to exposure. Low dose rat sleeping patterns were not disrupted. To measure activity, intact rats were exposed to 100 or 200 mg/kg per day for 14 days and were monitored for 6 days post-exposure. Rats in the high dose group displayed hyperactivity (i.e., increased crossings of cage) during the light period for four days following exposure as compared to olive oil exposed controls. High dose rats drank increased amounts of water during the dark period on the last day of exposure and for six days following exposure as compared to controls. Activity and water ingestion were not different from controls in the low dose group. Subsequently, rats were exposed to 200 mg/kg per day, again for 14 days. Rats were sacrificed in either the light or dark period on day 1 following exposure. Decreased levels of serotonin during both the light and dark periods were found in the frontal cortex, hippocampus and midbrain, possibly indicating decreased synthesis of the hormone. Decreased levels of serotonin's metabolite 5-hydroxyindoleacetic acid in the midbrain and hypothalamus allude to decreased turnover; this also occurred during the light and dark cycles. Hypothalamic levels of noradrenaline were increased during the dark cycle while increased turnover of noradrenaline, indicated by increased levels of its metabolite 3-methoxy-4-hydroxyphenylethyleneglycol, occurred in both the light and dark periods. Increased turnover of dopamine in the striatum during the dark period was exhibited as increased 3,4-dihydroxyphenylacetic acid and homovanillic acid levels. Insomnia and hyperactivity have previously been reported as the result of treatments leading to decreased levels of serotonin and its metabolite. The dopamine system is known to control angiotensin-induced drinking behavior; hypothalamic injection of noradrenaline also increased drinking in previous studies (Arito *et al.*, 1985).

Toluene was found to have no adverse effect on plasma butyrylcholinesterase activity in mice. Male and female NMRI mice were continuously exposed to toluene (99.5% pure) at 150 ppm for 30 days. The female mice had significantly decreased bodyweights and increased liver weights. Butyrylcholinesterase activity is regulated by the endocrine system; testosterone is mainly responsible for its activity level. Solvents such as TCE greatly increase the butyrylcholinesterase activities in male mice; a subsequent study revealed that TCE does not affect testosterone levels (i.e., synthesis and breakdown are not altered). As mice do not posses SHBG, which help protect testosterone from breaking down in the liver, TCE is not altering this mechanism either. TCE appears to affect butyrylcholinesterase independently of testosterone and may act on its own in either the liver or the pituitary/hypothalamus. Since toluene did not affect butyrylcholinesterase activity, it does not appear to decrease testosterone levels nor does it act directly in either the liver or the pituitary region on this particular enzyme (Kjellstrand *et al.*, 1985).

The neuroendocrine effects of toluene in drinking water were evaluated by Hsieh *et al.* (1990a). Male CD-1 mice were exposed to chromatography grade toluene (99.7% pure) at 17, 80 or 405 mg/l drinking water for 28 consecutive days (i.e., 5, 22 or 105 mg/kg bodyweight per day). Signs of toxicity and significant differences in food/water consumption were not present. The low dose group had significantly increased norepinephrine, dopamine and 3,4-dihydroxyphenylacetic acid, a dopamine co-metabolite, levels in the thyroid. Norepinephrine and serotonin levels in the midbrain were also increased. The mid dose group experienced increased norepinephrine levels, serotonin levels and turnover (increased metabolite 5-

hydroxyindoleacetic acid) and dopamine levels and turnover in the hypothalamus. Dopamine levels, serotonin levels and norepinephrine turnover were increased in the corpus striatum. Only an increase in dopamine turnover (i.e., co-metabolite homovanillic acid increase) was found in the cerebellum at the mid dose level. In the midbrain, the mid dose level resulted in increased serotonin and norepinephrine levels and turnover. The cerebral cortex of the mid dose group showed an increase in serotonin. Finally, the medulla oblongata results from the mid dose group included increased norepinephrine levels and turnover, serotonin levels and turnover, and dopamine turnover. The high dose group had lesser effects than the mid dose group with the exception of a dose-related increase in dopamine levels and norepinephrine turnover in the corpus striatum and an increased norepinephrine turnover in the cerebral cortex. Although the mid dose level clearly had the greatest effects, it is interesting to note that the low dose of 17 mg/l is near the EPA maximum contaminant level (MCL) of toluene in groundwater (i.e., 14.3 mg/l) in 1991. The MCL has been since decreased to 1 mg/l (ATSDR, 1994).

These results were confirmed in a study designed to determine the effects of toluene or benzene or both on neuroendocrine chemicals. As before, male CD-1 mice were exposed for 28 days to 80 or 325 mg chromatography grade toluene (99.7% pure) per liter tap water. Again, 80 mg/l had relatively greater effects than the higher dose level. Norepinephrine and dopamine levels were increased in the thyroid at the 80 mg/l dose level. In the corpus striatum, norepinephrine levels and turnover (increased levels of the metabolite vanillylmandelic acid), and dopamine turnover (increased homovanillic acid) were significantly increased. Dopamine levels and turnover (increased 3,4-dihydroxyphenylacetic acid and homovanillic acid), and serotonin turnover (increased 5-hydroxyindoleacetic) were significantly increased in cerebral cortex. Only serotonin turnover was increased in the midbrain. Norepinephrine levels, dopamine turnover and serotonin turnover were increased in the medulla oblongata at the 80 mg/l dose level. The high dose level caused an increase in serotonin levels and/or turnover in the hypothalamus, corpus striatum cortex, midbrain and medulla oblongata not seen in the lower dose group. The only other effect noted among high dose mice that was not present in low dose animals was an increase in dopamine turnover in the medulla oblongata (Hseih *et al.*, 1990b).

The immune effects of toluene in drinking water were evaluated in 1991 by Hsieh *et al.* Male CD-1 mice were exposed *ad libitum* to 17, 80 or 405 mg/l toluene in tap water for 28 days. There were no differences in bodyweight or food/water consumption between control and exposure groups. Overt clinical symptoms of exposure were not present. Immediately following exposure, the hypothalamic concentration of norepinephrine and its metabolite vanillylmandelic acid were significantly increased at all exposure levels. Serum adrenocorticotropic hormone levels following 28 days of exposure increased in a dose-response manner, although the increase was significant only in the high dose group. Serum corticosterone levels were measured prior to exposure and at 2, 7, 14 and 28 days during exposure; corticosterone levels were significantly increased as compared to controls in the high dose group on days 14 and 28 of exposure. Spleen lymphocyte-derived interleukin-2 synthesis was significantly decreased in the high dose group as compared to controls. Although controversial, norepinephrine has been reported to stimulate adrenocorticotropic and corticosterone production. Inhibited interleukin-2 and impaired immunocompetence have been reported as the result of elevated corticosterone levels, resulting in decreased T-cell proliferation.

Toluene Reproduction Studies

Yamada (1993) investigated the effects of lacquer thinner and its constituents, including toluene and xylene, on the reproductive organs of male Wistar rats. Rats inhaled toluene vapor twice a day for approximately four to six minutes (i.e., until the righting reflex was overcome) on seven consecutive days; the rats were sacrificed on the eighth day. Toluene had no effect on plasma testosterone levels, prostate acid phosphatase activity or testicular and accessory organ (i.e., epididymus, vas deferens, seminal vesicles and prostate) weights. Bodyweights and numbers of sperm in the epididymus were also not affected. Toluene did not interfere with the function of male rat reproductive organ function.

In an evaluation of the testicular effects of *n*-hexane, rats were also exposed to xylene or toluene. Sprague Dawley rats inhaled toluene at 1000 ppm for 18 hours daily for 61 days. The animals were observed for up to 14 months following exposure. One of eleven rats exposed to toluene alone had bilateral testicular alterations and decreased testicular weight one year post-exposure. Spermatozoa and germ cell immunoactivity were greatly decreased in this individual. Testicular weight, androgen synthesis, testosterone concentration, vas deferens morphology, spermatozoa morphology, noradrenaline concentration and germ cell line immunoreactivity to nerve growth factor were not affected in ten of eleven rats. Loss of immunoreactivity indicates a loss of germ cells. A combined exposure of toluene and *n*-hexane was found to significantly prevent the frequent occurrence of testicular atrophy and loss of germ cells induced by *n*-hexane alone; toluene has been shown to decrease the blood concentration of 2,5-hexanedione, the major metabolite of *n*-hexane (Nylen *et al.*, 1989).

Toluene was evaluated in the sperm-head abnormality test by Topham (1980). In this test, male mice (CBA males x BALB/c females) were intraperitoneally administered the test substance at fractions of the $LD_{50}$ for five contiguous days. Toluene doses were 0.1, 0.25, 0.5, 1.0 or 1.5 mg/kg per day. Caudal sperm were collected and evaluated five weeks post-exposure. A positive response would include at least double the rate of SHA occurrence in the negative control group and also statistical significance at $p<0.05$. The SHA evaluation was negative. The highest toluene dose was found to be lethal. This test has found use in identification of substances which cause transmissible genetic damage by disrupting the process of spermatozoa differentiation.

Toluene Developmental Studies

Pre-Mating/Gestational Exposure

Donald *et al.* (1991) reviewed a rat inhalation study beginning 80 days prior to mating and continuing through to gestation day 19. CR.CD dams were exposed to 100, 500 or 2000 ppm toluene (99.99% pure) 6 hours daily through the exposure period while sires were exposed to 2000 ppm. On gestation day 21, fetal bodyweights in the high dose group were significantly decreased. Maternal toxicity was not present. An insignificant increase in skeletally-retarded fetuses was present in the high dose group. The NOAEL for this study was 500 ppm (International Research and Development Corp., 1985).

Gestational Exposure

The teratogenicity of high concentrations of toluene was determined in Sprague-Dawley rats. Dams were exposed to 500, 1000, 2000, 3500 or 5000 ppm toluene (99% pure) for 6 hours daily on gestation days 6 through 15. One mortality occurred in the high dose group. Narcosis was noted in both the 3500 and 5000 ppm animals. Maternal bodyweight gain and food consumption decreased while water intake increased in the 2000 ppm and greater exposure groups. Pre-implantation and resorption losses occurred significantly more frequently among the high dose group. Although fetal bodyweights were decreased in the 3500 and 5000 ppm groups, no malformations were found. In a subsequent portion of this study, rats were exposed in the same manner to 250, 750, 1500 or 3000 ppm toluene. A single death occurred at the high dose. Maternal bodyweight again decreased in the two highest dose levels (i.e., 1500 and 3000 ppm); salivation and lack of coordination were noted among these animals. Food consumption was decreased at the high dose. Maternal relative liver weights were significantly increased over controls with exposures at 750 ppm and higher. No fetal malformations were found; however, growth retardation (i.e., decreased fetal weight and sternebrae ossification) occurred at 1500 and 3000 ppm. The results indicate that toluene is not a selective developmental toxicant and is only fetotoxic at maternally toxic doses (Roberts *et al.*, 1993).

Similar doses during a different stage of gestation yielded similar results. Rats were exposed to 300, 900, 1200 or 1400 ppm toluene for 6 hours daily on gestation days 9 through 20. Examination on day 21 revealed an increase in resorption among the 1200 and 1400 ppm exposure groups. Fetal weight was decreased in all toluene exposed animals in a dose dependent manner. Skeletal malformations were not found. Delayed ossification, related to reduced fetal weight, was found in the 1200 and 1400 ppm groups. Although additional lumbar ribs occurred more frequently among toluene exposed fetuses, the occurrence was not dose related. Toluene was not found to be teratogenic or fetotoxic; growth retardation at the highest doses was found (Hartmann *et al.*, 1994).

A NOAEL of 600 ppm was found in a teratogenicity study with Sprague-Dawley rats. Dams were exposed 6 hours daily on days 7 through 17 of gestation to 600 or 2000 ppm toluene. The high dose resulted in decreased maternal and fetal weight gain, increased fetal mortality and embryonic growth retardation. No external, internal or skeletal malformations were found. Behavioral evaluations before and after weaning showed no significant difference between exposed pups and air exposed controls. No toxic effects on dam or fetus were found at 600 ppm (Ono *et al.*, 1995).

CFY rats exposed to toluene at various stages of gestation showed embryotoxicity, but not teratogenicity, in the 1978 Hudak and Ungvary study. Rats continuously exposed to 1500 $mg/m^3$ (399 ppm) toluene on days 1 through 8 of gestation had embryos with significantly increased skeletal retardation, shown by poor ossification of sternebrae, bipartite vertebral centers and shortened 13th ribs. Mean fetal weights were also significantly decreased and the proportion of weight retarded fetuses subsequently was increased. Two of the exposed rats died. Animals exposed under the same conditions on days 9 through 14 of gestation demonstrated statistically significant fetal skeletal anomalies of fused sternebrae and extra ribs. Fetal and maternal weights were not affected. CFY rats exposed to 1000 $mg/m^3$ for 8 hours per day over days 1 through 21 of gestation had significantly increased occurrence of fetal skeletal retardation. This exposure was maternally lethal in 5 of the 14 animals.

Toluene abuse was modeled in a Sprague-Dawley rat study by Gospe *et al.* (1994). Rats were exposed daily via gavage to 520 mg toluene/kg bodyweight on gestation days 6 through 19. This dose was calculated to produce blood toluene levels similar to reported blood values for a toluene abuser exposed for 3 hours to 3290 ppm; abuse concentrations are reported to range between 4000 and 12000 ppm. Dams and fetuses were sacrificed two hours after the last exposure. This dose was not maternally lethal; however maternal weight gain was significantly decreased as compared to controls. Toluene exposure did not effect the number of stillbirths or implantations; major malformations were not increased. However, placental and fetal weights were both significantly decreased as compared to controls. Absolute kidney and liver weights were significantly decreased. Relative organ weights were not affected, except for relative brain weights which were significantly increased; no change was noted in the encephalization index (i.e., brain weight/bodyweight$^{0.88}$). Overall, a generalized growth retardation was seen in fetuses exposed *in utero* to relatively low abuse levels of toluene. The increased relative brain weights were likely the product of the reduction in fetal bodyweight and not teratogenicity, as indicated by the insignificant encephalization index value.

The dual effects of toluene exposure and malnourishment were evaluated by da Silva *et al* (1990a). Well nourished (i.e., allowed food *ad libitum*) and malnourished (i.e., fed 50% of well nourished intake) Wistar rats were exposed to 1.2 g toluene/kg bodyweight subcutaneously on days 8 to 15 of gestation. Both well nourished and malnourished controls were exposed to the corn oil vehicle alone. Fetuses were examined on day 20 of gestation. Toluene exposed dams had significantly decreased bodyweight gain during exposure as compared to well nourished controls; this decrease was not significant over the entire gestation period. No other maternal toxicity signs were observed. Stillbirths, implants, malformations and fetal and placental weights were not affected by toluene exposure. However skeletal growth retardation, measured as decreased numbers of ossification centra, was found among malnourished treated rats as compared to malnourished controls, but not among well nourished treated rats as compared to well nourished controls. A subsequent study examined the effect of toluene administered on days 14 through 20 of gestation. The rats were allowed to deliver naturally and all litters were fostered out to well nourished, untreated surrogates. Again, maternal weight gain during gestation was decreased with toluene exposure. One malnourished treated dam died in labor and another delivered all dead fetuses; a well nourished control dam cannibalized her pups. Toluene exposure was found to decrease birth weight of both male and female pups; malnourished treated pups were significantly lighter than both the well nourished and malnourished controls. Weight decreases persisted in 95-day old treated males as compared to well nourished male controls. Toluene exposure and malnourishment decreased absolute brain weights. Relative brain weights were increased in simple malnutrition; this ratio was not different in toluene treated rats as compared to controls. The brain sparing phenomenon allows normal brain development during malnutrition. Skeletal growth retardation was again found in treated fetuses. Toluene treatment did not affect spontaneous activity as measured in a motility box at 30 days of age. Conditioned two-way shock avoidance measured in an automatic shuttle box at 95 days old was also not affected by toluene exposure. A concurrent study measured toluene blood levels in non-pregnant female rats. Toluene (1.2 g/kg) was administered subcutaneously and blood was collected at 2, 4, 6, 12 and 24 hours post-exposure. Toluene blood levels were not different between well and malnourished rats. Blood levels correlated well with levels observed in volunteers exposed to 400 ppm toluene for 8 hours.

In Donald *et al.* (1991), a rat inhalation teratogenicity study by Tatrai *et al.* (1980) was reviewed. CFY dams were exposed to 266 ppm continuously on days 7 through 14 of gestation. Maternal and fetal weights were not adversely affected. However, maternal relative liver weights were significantly increased. A significant increase in skeletally-retarded fetuses, as compared to the rate in air exposed controls, was the major effect noted.

In the same review, a similar study by Litton Bionetics (1978a) was also detailed. CRL:COBS CD (SD) BR rats dams were exposed via inhalation to 100 or 400 ppm toluene for 6 hours daily during gestation (days 6 through 15). Maternal and fetal weights were not affected by exposure. Growth retardation was not found and evidence for teratogenicity was not present at either concentration (Donald *et al.*, 1991).

Rats exposed to 800 mg/m$^3$ toluene *in utero* were found to have altered behavioral patterns. Dams were exposed to toluene six hours daily on gestation days 14 through 20. Toluene exposure resulted in increased litters with low birth weight pups. Exposed male pups had shorter latencies than control males in choosing one side of a T maze during the spontaneous alternation test. Toluene was found to be fetotoxic and to effect exploratory behavior, which is thought to be linked with hormonal disruption in early life (da Silva *et al.*, 1990b).

In the same study, hamsters were exposed to 800 mg/m$^3$ toluene for 6 hours on days 6 through 11 of gestation. Rotarod performance was significantly impaired among toluene exposed hamsters. Toluene was found to have neuromotor effects in the hamster (da Silva *et al.*, 1990b).

In a review of the teratogenicity of solvents, Schardein (1993) reported mixed results for toluene. This review cited a 1977 study by Hudak *et al.*, in which toluene was found non-teratogenic in rats. However, a 1979 study by Nawrot and Staples found toluene to be teratogenic to mice. A review by Donald *et al.* (1991) detailed this study after personal communication with Staples. CD-1 mice were orally exposed 3 times daily to 0.9, 1.5 or 3.0 mg/kg per day on gestation days 6 through 15 or to the high dose only on days 12 through 15. Maternal toxicity was reportedly absent. Fetal bodyweights were significantly decreased in the mid and high doses if exposed on gestation days 6 through 15. Offspring exposed on days 12 through 15 were not different from controls in weight. A significant increase in cleft palate was noted in the high dose group exposed on days 6 through 15.

A mouse inhalation study reported teratogenicity but not fetotoxicity. CD-1 mice were exposed to 200 or 400 ppm (750 or 1500 mg/m$^3$) toluene 7 hours per day from days 7 to 16 of gestation. For the prenatal study, mice were sacrificed on day 17. Exposure to 200 ppm resulted in the occurrence of dilated renal pelvis, possibly indicating desynchronization of the normal maturation process. The high dose caused fetal rib malformations. Dams of the high dose group exhibited increased lactate dehydrogenase activity in the brain; relative liver weights of dams in the low exposure group were significantly reduced. Nonpregnant mice exposed concurrently exhibited elevated LDH activities of the liver and kidney at both doses and the high dose, respectively. Organ damage can result in increased LDH. A postnatal study was conducted simultaneously. Dams were exposed to 400 ppm (1500 mg/m$^3$) for 6 hours daily over days 7 through 16 of pregnancy. The study was terminated on postnatal day 21. A significant increase in neonatal bodyweight on the first postnatal day was observed (Courtney *et al.*, 1986).

In his 1991 review, Donald *et al.* described a mouse teratogenicity study by Shigeta *et al.* (1981). ICR dams were exposed to 100 or 1000 ppm toluene for 6 hours daily throughout gestation (days 1 through 17). A significant increase in extra 14th ribs was noted among fetuses exposed *in utero* to the high dose.

Teratogenicity was suggested in a study evaluating ICR mice exposed to toluene throughout gestation. Mice were exposed via inhalation to 100 or 1000 ppm toluene from day 1 through day 17 of gestation. Two-thirds of the fetuses were examined on day 18. Resorption rates were slightly, but not significantly, increased among exposed dams. The number of implantations, litter size and fetal bodyweights were not affected. External malformations were also not different from controls. However, skeletal abnormalities including extra 14th ribs and rudimentary 14th ribs were significantly increased among high dose animals as compared to controls. The remaining third of the fetuses were allowed to develop; bodyweight gains and development, including eye opening, ear unfolding, hair coat and coordinated walking, were not affected by toluene exposure. The incidence of skeletal abnormalities at 1000 ppm suggests teratogenicity at this level (Shigeta *et al.*, 1982).

CFLP mice exposed continuously over days 6 through 13 of gestation to 500 $mg/m^3$ of toluene exhibited significantly decreased mean fetal weight, an increased percentage of weight retarded fetuses and increased skeletal anomalies. Skeletal anomalies such as fused sternebrae and the presence of extra ribs are not considered teratogenic but embryotoxic. A continuous exposure of 1500 $mg/m^3$ for the same duration in these mice was universally maternally lethal (Hudak and Ungvary, 1978). In a later study, CFLP mice were exposed to 500 or 1000 $mg/m^3$ toluene for 3 four-hour sessions daily on days 6 through 15 of gestation, resulting in significant increases in growth retarded fetuses (Ungvary and Tatrai, 1985).

Similar exposures in New Zealand white rabbits were also evaluated. Rabbits were exposed continually to toluene at 500 or 1000 $mg/m^3$ over days 7 to 20 of gestation. Dams in the 1000 $mg/m^3$ group responded with significantly decreased percent weight gain combined with a significant increase in percent relative liver weight. The embryotoxic effects seen in this study indicate a possibility for "spontaneous" abortions in occupationally exposed women (Ungvary and Tatrai, 1985).

Klimisch *et al.* (1992) conducted rabbit prenatal inhalation studies in order to enable the proposal of an occupational pregnancy guidance value. In the first study, groups of 15 Himalayan rabbits were exposed to 30, 100 or 300 ppm toluene 6 hours daily on days 6 through 18 post-insemination. For the second study, groups of 20 rabbits were exposed to 100 or 500 ppm. All rabbits were sacrificed 29 days post-insemination. Gestational parameters including maternal and fetal weights were within the variation range of this strain in both studies. Maternal toxicity was not found. A significant increase in number of fetal soft tissue variations including separated origin of carotids was found in the second study in both the 100 and 500 ppm groups. The incidence was not dose dependent and these variations were not significant in the first study. Similarly, an increase in irregularly shaped sternebrae was noted among the first study's 100 ppm group that was not found in the higher or second dose groups. Fetal skeletal retardations including small, incomplete or non-ossified sternebrae were found in all dose levels of the first study. Although significant, the results were not dose dependent nor were significant effects seen in the second study. Due to the lack of dose-response and reproducible effects, the authors determined that any variations in these studies were incidental and that the NOAEL for maternal and prenatal rabbits is 500 ppm. Using rat and mice NOAELs

and LOAELs from other studies, an occupational pregnancy guidance value (i.e., 8 hours/day) of 20 ppm was suggested.

Gestational/Postnatal Exposure

Toluene was found to affect learning abilities of high avoider rats. Tokai High Avoider rats were exposed to 100 and 500 ppm toluene for 7 hours daily from day 13 of gestation through postnatal day 48. Low dose fetuses had slightly higher bodyweights as compared to high dose and control fetuses. Physiological development, including ear unfolding, primary hair coat, incisor eruption and eye opening, reflex and locomotor activity were not different from controls. The Sidman avoidance test was administered on postnatal days 49, 100 and 150 days of age for a duration of ten days each time. Female toluene exposed offspring had lower avoidance rates than female controls during the first half of the Sidman test at 49 days of age. Female test results were not different from controls at any point thereafter. Male toluene exposed offspring had significantly lower avoidance rates and higher individual avoidance variability than the male controls when tested at 49 days. Toluene exposed males had noticeably lower rates of avoidance at 100 and 150 days as well (Shigeta *et al.*, 1986).

A two-generation rat study was reported by Thiel and Chahoud in 1994. First generation pregnant Wistar rats were exposed to 300, 600, 1000 or 1200 ppm for 6 hours daily on gestation days 9 through 21. No difference in mating, fertility and pregnancy indices were found when compared to controls. Litter size was not affected. However, postnatal survival was reduced in the high dose group. Mean fetal weight was decreased significantly among 1000 and 1200 ppm animals; physiological development including eye opening, incisor eruption, ear unfolding and vaginal opening was delayed in these animals. Behavioral tests were not different from controls. Second generation females were similarly mated and exposed as their dams had been. Again, litter size was not different. However, postnatal survival was reduced in both the 1000 and 1200 ppm groups; ear unfolding, incisor eruption and eye opening were delayed. Behavioral tests did not show toluene induced effects. The authors suggest a NOAEL of 600 ppm in Wistar rats.

Continuous pre- and postnatal exposure of mice to toluene in drinking water resulted in behavioral effects. Nya:NYLAR dams were exposed to 16, 80 or 400 ppm analytical grade toluene in distilled water from the first hour of the mating period through the weaning of the pups (21 postnatal days); offspring continued exposure through behavioral testing (55 postnatal days). Volumes of water consumed did not differ between control and treatment groups. No effect on mortality, abortion or cannibalization rates were seen. Bodyweight gain and development of eye and ear openings also were not affected. The body righting reflex, tested at 7 days of age, and startle response (i.e., response to a click behind the ear), tested on postnatal days 13 and 14, were not different from controls. A deficit in habituation was exposed in open field testing at 35 days of age; this dose-related deficiency was significant only in the 400 ppm exposure group. Additionally, impairment of rotorod performance was seen in all dose groups at 45 through 55 postnatal days; the impairment was not dose related. Toluene exposed animals had shorter times on the rotorod as compared to the control times for the first three of four trials; the times during the fourth trials were not different from controls. An abnormal splay of the hindlimbs was noted during the fourth trial in the treated rats, suggesting an adaptation to overcome the deficiency (Kostas and Hotchin, 1981).

Toluene Lactation Studies

No neurobehavioral effects were found among rats exposed to toluene through nursing. Lactating Wistar rats were subcutaneously injected with 1.2 g/kg toluene daily on lactation days 2 (the day after birth) through 21. Neurosomatic development and exploratory behavior, evaluated prior to weaning, were not different among toluene exposed pups as compared to vehicle (i.e., corn oil) exposed controls. Open field behavior, tested on postnatal day 35, and shuttle box performance as adults were likewise not affected. Toluene levels in milk were determined through a separate study in which a single injection occurred on day 10 of lactation. Four hours after exposure, toluene levels in milk were five times higher than blood levels (da Silva *et al.*, 1991).

Toluene Postnatal Studies

A postnatal exposure by Slomianka *et al.* (1990) examined the effect of 100 or 500 ppm toluene on the developing hippocampal region of the cortex. Wistar rat litters and foster dams were exposed for 12 hours daily on postnatal days 1 through 28. The layers of Ammon's horn and the subiculum, two major areas of the hippocampus, were not affected by exposure. However, specific neuron and terminal field layers of the area dentata, the final major area of the hippocampus, were significantly decreased in volume in a dose-response manner as compared to controls. Although several layers were significantly decreased in volume at the 100 ppm dose level, the volumes were often within clinically normal ranges. The hippocampus has been found to be affected in a similar manner in adult rats at higher doses.

Neonatal exposure to toluene was found to have immediate and long-lasting catecholamine effects. Male Sprague-Dawley rats were exposed to 80 ppm toluene 6 hours daily on postnatal days 1 through 7. At eight weeks of age, treatment and control rats were exposed to air six hours daily over three consecutive days. Sacrifice occurred 16 to 18 hours post-exposure. In order to measure catecholamine turnover, a portion of the control and exposed groups were administered the tyrosine hydroxylase inhibitor alpha-methyl tyrosine methyl ester hydrochloride two hours prior to sacrifice. Toluene exposure resulted in decreased dopamine levels and corresponding increased dopamine turnover in one area of the forebrain. Decreased catecholamine turnover was found in some hypothalamic areas as compared to controls. The substantia nigra, an area of the midbrain, was characterized by increased dopamine turnover and decreased noradrenaline levels with the accompanying increase in noradrenaline turnover. Inhibitor administration to control animals resulted in increased prolactin secretion; toluene exposure prevented this secretion significantly. For the second portion of the study, rats were again exposed neonatally to either air or toluene. At 8 weeks of age, this second group was exposed to toluene at 80 ppm for 3 six-hour periods. Air exposed neonates which were exposed to toluene as adults served as positive controls. Forebrain dopamine levels increased in one area, while dopamine turnover was decreased in the same and other regions, as compared to positive controls. Increased catecholamine turnover occurred in several hypothalamic areas. The effects in the substantia nigra were also reversed, with decreased dopamine turnover and increased noradrenaline levels due to decreased noradrenaline turnover. Prolactin levels were not affected by inhibitor administration after toluene exposure. Neonatal toluene exposure appears to have made permanent changes in catecholamine receptors, even to the point of reversing subacute effects of toluene administered to adult rats. Major alterations of serum hormones were not seen after neonatal, adult or neonatal/adult

exposures to toluene, indicating the neuroendocrine system remains largely intact (von Euler *et al.*, 1989).

The effects of neonatal toluene exposure on liver enzymes and serum hormone levels were evaluated in a 1985 study by Hansson *et al.* Sprague-Dawley dams and litters were exposed to 80, 500 or 1000 ppm analytical grade toluene for 6 hours daily over postpartum days 1 through 7. Rats were weaned on day 21; sacrifices occurred on days 8, 21 and 56. At postpartum day 8, male and female offspring had significantly decreased bodyweights at the mid and high exposure levels. Males and females had decreased absolute liver weights at the high exposure while females in the low and mid doses had increased absolute liver weights. Relative liver weights, however, were increased for males at the low and mid, and females at all exposure levels. At 56 days, bodyweights had recovered and male offspring in the low and mid exposure groups were even significantly larger than controls. Absolute liver weights of both sexes in the low exposure group were increased as were the relative liver weights in this group. Males in the high exposure group also had increased relative liver weights. Liver enzyme effects on day 8 were mostly in male offspring. Cytochrome P-450 levels were increased in a dose dependent manner, as was 7-ethoxyresorufin O-deethylase activity in the mid and high exposure levels. Aryl hydrocarbon hydroxylase (AHH) activity was decreased among the low dose animals but increased in the mid and high dose groups. Females only responded with increased 7-ethoxyresorufin O-deethylase activity at the high exposure. At 56 days, males had significantly decreased AHH activity at the mid exposure level while females displayed increased P-450 levels in the high exposure group. Androstenedione metabolites on Day 8 showed decreased P-450 dependent metabolism among both sexes of the low exposure group. However, P-450 dependent metabolites were significantly increased for both sexes in the higher dose groups. Reduction metabolites independent of P-450 were not affected except for a slight but significant decrease in the mid dose females. At 21 days, low exposure males were found to have decreased levels of one P-450 dependent metabolite while independent metabolites for both sexes were increased in the high exposure group. Mid dose males at 56 days had the lone significant effect of increased P-450 independent metabolites. Serum levels of testosterone in males were significantly affected by toluene exposure. On Day 8, decreased testosterone levels were found at all dose levels. At 21 days, the mid and high dose males still had decreased levels and the decrease persisted in high dose males at 56 days. Other male hormone levels and female hormone levels were not affected. Overall, as toluene tended to decrease serum testosterone levels, an interference with masculine differentiation in the male rat brain is suggested, which would therefore affect synthesis and/or metabolism of androgens. As AHH activity and P-450 independent metabolism are both sex differentiated, the changes observed in these parameters for exposed males support this suggestion.

Toluene *In Vitro*/Screening Studies

Toluene was found to decrease sperm motility, reduce fertilization and cause embryo degeneration *in vitro*. B6D2F1 mice were used for this three part experiment. To measure sperm motility, caudae epididymides were removed from male mice and incubated in BMOC-3 media for four hours. Medium concentrations of toluene were 0.0867, 0.867, 8.67 and 86.7 µg/ml. Significantly decreased motility, as compared to control sperm, was seen in the 0.867 µg/ml and higher concentrations throughout the 4 hour test period. *In vitro* fertilization rates were measured using sperm prepared as above and incubated for only one hour. Cumulous masses were removed from superovulation-induced mice and were placed in media of the

same varied concentrations, after which sperm incubated in the appropriate media concentrations were added. Fertilization was assessed after incubating 24 hours. Fertilization rates were significantly decreased in the 8.67 and 86.7 μg/ml concentrations as compared to control rates. Finally, to assess preimplantation embryo toxicity, embryos were collected 24, 48 and 60-72 hours (2, 4 and 8 cell embryos, respectively) after artificial insemination. Embryos were incubated in 0.0867, 0.867 or 8.67 μg toluene/ml media. A significant degeneration rate was noted only in the 8.67 μg/ml concentration. The authors report toluene levels in municipal groundwater to be as high as 11 μg/l (i.e., 0.011 μg/ml) (Yelian and Dukelow, 1992).

An *in vitro* study using 10.5 day old rat embryos incubated in toluene solutions for 40 hours found a dose dependent increase in embryotoxicity. The NOEL solution concentration was 1.46 μmol/ml. At 2.25 μmol/ml, embryo growth, as measured by crown-rump length and protein content of the embryos, was significantly decreased as compared to controls. Development, as measured by somite numbers, and yolk sac diameter were also significantly decreased. Growth and development were more impaired at 4.06 μmol/ml, in a dose-effect manner. Severe growth retardation effects included decreases of more than two standard deviations in the size of the telencephalon as compared to embryos of the same crown-rump length (Brown-Woodman *et al.*, 1994b). The telencephalon includes the cerebrum, basal ganglia and the limbic system (McCance and Huether, 1990). Embryos incubated in toluene for only the first 16 hours of the 40 hour culture period showed similar growth, development and yolk sac decreases, including a greater decrease in protein content than the 40 hour embryos as compared to controls. A mixture of toluene and xylene together produced additive effects in embryos incubated for 40 hours. *In vitro* incubation concentrations are relative to serum concentrations. The published maximum permissible occupational blood concentration of toluene is 0.011 μmol/ml, allowing a safety factor of 100 over the NOEL in this study. However, embryos exposed to toluene at different times of gestation or throughout gestation may have a different NOEL (Brown-Woodman *et al.*, 1994b).

A previous, similar study by the same researchers did not produce a NOEL. Sprague-Dawley rat embryos were explanted at 9.5 days and incubated in 0.1, 0.5 or 1.0 μl/ml (initial concentration) toluene for 48 hours. At 0.1 μl/ml, crown-rump length, somite number and yolk sac diameter were all significantly decreased as compared to control embryos. These growth and developmental parameters decreased in a dose dependent manner with the higher concentration exposures. The industrial maximum permissible blood level for toluene is 0.001 μl/ml; blood toluene levels measured from glue-sniffing adolescents with neurological impairment ranged from 0.0008 to 0.008 μl/ml and postmortem blood levels following acute toluene exposure ranged from 0.054 to 0.077 μl/ml. Therefore, blood toluene levels high enough to cause the studied level of embryotoxicity would likely be maternally toxic (Brown-Woodman *et al.*, 1991).

Chick embryos exposed to toluene through injection into the air space of the egg showed slightly increased rates of teratogenicity. White Leghorn SK 12 eggs were injected with 5, 25, 50 or 100 μmol of toluene dissolved in olive oil on days 2, 3 or 6 of incubation. Embryos were examined on day 14. The toluene $LD_{50}$ was greater than 100 μmol per egg. Embryotoxicity increased in a dose-response manner when toluene was administered on the sixth day; comparatively little embryotoxic effect was seen at any dose level given on the second day. The high dose given on day 6 of incubation resulted in universal embryo death. Dead embryos from lower doses tended to be longer than dead embryos from higher doses, indicating delayed

toxicity from lower doses. The toluene malformation frequency (3/46 embryos of 6.5%) was roughly twice that of the olive oil controls (2/56 or 3.6%). Malformations included profound edema and skeletal abnormalities. Control malformations were eye and skeletal abnormalities. Statistical relevance was not calculated in this study (Elovaara *et al.*, 1979).

Toluene was found to have an A/D (adult/developmental) minimal effective concentration ratio of 1.7 when evaluated in the hydra developmental assay. This was comparable to A/D ratios calculated from published mammalian studies (>1 - ≤3). Ratios less than 3 are generally not differentiated from each other; priority for further testing (i.e., mammalian testing) goes to chemicals with ratios greater than 3 or those greater than 1 with widespread human usage and potential impact (Johnson *et al.*, 1988).

Toluene Epidemiological Studies

Male rotogravure printers exposed to toluene were found to have low plasma hormone levels. Workers from two printing companies were exposed to occupational levels of toluene below the current TLV time weighted average (TWA of 80 ppm) at the date of the study. After a week of environmental monitoring of each job type in each plant, an average exposure concentration was calculated for each exposed printer. Individual concentrations fit into six levels of exposure; the average exposures for printers in each plant were 11 and 47 ppm. Post-shift blood concentrations of toluene ranged from 0.19 to 8.0 μmol/l. The control group consisted of craftsmen and of workers from a metal industry; neither group had exposure to organic solvents. No statistical differences in plasma hormone levels were detected between the total exposed group and the controls. However, when heavy drinkers were excluded and the exposed group was sorted by age, the researchers found significantly decreased levels of LH, FSH and testosterone in the blood, as compared to age-matched controls. These results indicate an effect within the CNS or the hypothalamic-pituitary axis. Additionally, exposed workers (minus the heavy drinkers) had significantly increased serum ALP levels when comparing liver function tests with controls (Svensson *et al.*, 1992a).

In a later study, serum hormone levels were again found to be altered among a different set of 20 rotogravure printers exposed to toluene. The printers were from one company; the mean employment duration and mean age of these men were 25 and 48.2 years, respectively. Workers from a margarine and gelatin factory served as a cohort without toluene exposure; the mean age of these 44 men was 39.0 years. None of the printers had confirmed solvent toxic encephalopathy, even after ten long term employees were evaluated through psychometric testing. The TWA, after 5 days of personal sampling, was found to be 36 ppm with a range of 8 to 111 ppm. The median blood toluene level was 1.7 μmol/l; 5.7 mg/kg was the median subcutaneous fatty tissue level. Cohort blood levels rarely exceeded the detection limit (i.e., 6 of 21 men sampled) with the highest level reaching 0.1 μmol/l. A cumulative exposure index, calculated based on historical exposure data from major printing companies in Sweden, was estimated at 5630 ppm-years. Printers had significantly decreased serum FSH, luteinizing hormone and free testosterone levels as compared to cohorts. Free triiodothyronine levels were increased. FSH and luteinizing hormone levels remained significantly decreased after age stratification of the exposed and cohort groups. Blood toluene levels of both groups had a significant negative correlation to serum prolactin levels; blood toluene levels were found to have a greater influence than age on prolactin in a multiple regression analysis. After four

weeks vacation, eight printers were found to have significantly higher serum FSH and luteinizing hormone levels than prior to vacation. Blood toluene, serum TSH, free triiodothyronine and free thyroxine levels were significantly decreased. While hormonal differences were significant, the majority of serum values fell within reference limits. The effects appear to be reversible and therefore sub-acute in nature; the non-correlation between the cumulative exposure index and hormone levels support this assumption (Svensson *et al.*, 1992b).

Women working in a factory situation with high (50 to 150 ppm, mean 88 ppm) exposure to toluene were compared to women in the same factory with low (0 to 25 ppm) toluene exposure, to women in the external community but of the same socioeconomic group, and with their own menstrual histories. No significant differences were found for alterations in regularity or duration of menstrual cycles. A significant difference was found between the highly exposed women and the community group in frequency and severity of dysmenorrhea (difficult and painful menstruation); there were no differences between the two factory groups. Also, no significant differences were found in the menstrual histories (before and after employment comparison of individuals' menstrual abnormalities) of either factory group. No clear association of toluene exposure and menstrual abnormalities was found (Ng *et al.*, 1992).

Goodwin (1988) reported the case histories of five pregnant women with nonspecific abdominal pain or general weakness as a result of paint sniffing. These five cases presented to a clinic over a period of three years. Each woman had decreased arterial pH, decreased serum bicarbonate and increased serum chloride levels along with high urine pH. Four of the five fetuses had normal ultrasound and heart tracings; Apgar scores were normal at birth. The fifth had a deficient amount of amniotic fluid and variable heart tracings, prompting a cesarean delivery; Apgar scores were low at one and five minutes. Fetal hyperchloremic acidosis was noted in each of the five infants. One infant had a normal birth weight and no gross anomalies. Three infants were growth retarded; one had facial similarities to fetal alcohol syndrome. The cesarean delivery was found to have ventricular septum, ear and jaw defects as well as hydronephrosis or dilation of the kidneys due to urine flow obstruction. The severity of effects was found to be loosely related to self-reported habitual exposure amount (range of 0.5 to 2 cans of paint/day) and duration (range of 0.5 to 6 years).

Hersh *et al.* (1985) described three children exposed *in utero* through toluene abuse. Each mother inhaled primarily pure toluene paint reducer throughout pregnancy, with occasional use of spray paint. Each child had significantly decreased weight, length and head circumference, which persisted at three or four years of age. Facial anomalies included short eyelid slits, deep-set eyes, a small midface accompanied by a flat nasal bridge and small nose in two of the children, low-set ears and a small lower jaw. Blunt fingertips and small fingernails were apparent in all three. Delayed mental development with language difficulties and hyperactivity was ubiquitous. Structural abnormalities of the urinary tract were found in two of these children. These anomalies are reminiscent of fetal alcohol syndrome and suggest that toluene is teratogenic.

In a later report, Hersh (1989) described two more children whose mothers had abused toluene paint reducer prior to and throughout pregnancy. Weight, length and occipitofrontal head circumference were not different from normal at birth; however, at two to three years, these parameters were found to be at the tenth percentile or lower. Both children had the facial anomalies mentioned above including the flat nasal bridge and small nose. Although both

children had blunted fingertips, only one had small fingernails. Delayed development, language deficits and short attention span was apparent in one child while the other suffered only a mild expressive language impairment. Urinary tract ultrasounds were normal. These two children add weight to the embryopathy of toluene abuse.

Pearson *et al.* (1994) evaluated 18 infants referred to her program through maternal admission of prenatal toluene abuse or through evidence of toluene abuse at delivery. Each mother was a regular abuser of spray paint. Seven of the 18 infants were premature; one was stillborn. Six infants were found to be small for their gestational age while five were born with microcephaly. Dysmorphic facial features associated with toluene abuse were apparent in as many as four (small nose) to ten (short eyelid slits and small lower jaw) infants. Abnormally small fingernails were found in five babies. Renal ultrasound performed on 13 of the infants revealed abnormalities in 4. Only nine infants were available for re-evaluation three months to three years after birth; growth deficiency remained only in three of the children but microcephaly was significant in eight of the nine subjects. Six children underwent developmental evaluation; a developmental delay was apparent in five. A study bias likely exists as some children were referred to the program postnatally, selecting for children with more obvious outcomes of toluene abuse.

Arnold *et al.* (1994) reviewed another 35 cases of *in utero* toluene exposure. The pregnancies of 15 women were evaluated; the complications of 21 pregnancies among these women have been reported previously but are not examined elsewhere in this paper. The women were identified from hospital records of prenatally drug exposed children; sibling records were subsequently evaluated for evidence of toluene exposure. Controls were selected by identifying the two same-sex infants born at the study hospital immediately before and after the toluene exposed infant. The racial distribution of the control and exposed populations were similar. Of the 35 pregnancies, 3 resulted in perinatal death. The occurrence of prematurity, decreased birth weight and length less than the tenth percentile and small head circumference (42, 52, 24 and 32%, respectively) was significantly increased as compared to controls. Only three infants were noted to have dysmorphic features; consistent were the short eyelid slits, small midface and small nose. Renal abnormalities were not apparent. As infants, seven babies were reported as irritable or jittery. Re-evaluation was performed on 24 children; most were one year old or older. Weight and height less than the fifth percentile and microcephaly occurred in 46, 38 and 46% of the children, respectively. Low weight and microcephaly were significantly more common among children born to mothers who had abused toluene for four years or longer, as compared to controls. Developmental assessment was performed on 14 of the 24 children. Cognitive or motor delay and speech delay were noted among 38%. Behavioral disturbances reported among these toluene exposed children included hyperactivity, head banging and aggressiveness.

## Ethylbenzene

Few studies on ethylbenzene were located in this literature review; the studies found provide limited evidence of endocrine activity with effects similar to those reported for toluene. Ethylbenzene was found to produce changes in brain neurotransmitter levels. Although reproductive organ cells were not specifically targeted after ethylbenzene inhalation exposure, fetotoxicity without teratogenicity was the developmental outcome in the studies found. The need for further studies, including mechanistic experimentation, is indicated.

Ethylbenzene Toxicity Studies

In a 1992 NTP report, Chan investigated the effects of ethylbenzene (99% pure) inhalation in F344/N rats. Male and female rats were exposed to 100, 250, 500, 750 or 1000 ppm for 6 hours daily, 5 days a week for 13 weeks. These concentrations were not found to be lethal. A slight decrease in weight gain at the high dose was found to be insignificant. However, absolute and relative lung, liver and kidney weights were increased in both sexes. No changes in sperm or vaginal cytology were found.

In the same study, B6C3F1 mice were also evaluated. Again, no animals died during exposure. Mouse bodyweights were not affected; only increased liver weights were found. As in rats, no changes were observed in vaginal cytology or sperm morphology (Chan, 1992).

Ethylbenzene Neuroendocrine Studies

Exposure of male Sprague-Dawley rats to high levels of ethylbenzene and commercial xylene resulted in changes of neuroendocrine levels in parts of the brain. The rats were exposed for 6 hours per day for 3 days to 2000 ppm of either ethylbenzene or commercial xylene (2% *o*-, 64.5% *m*- and 10% *p*-xylene, 23% ethylbenzene and 0.5% benzene). Neuroendocrine levels were measured 16 to 18 hours post-exposure. Ethylbenzene exposure resulted in significant decreases in noradrenaline levels within regions of the hypothalamus and significant increases in catecholamine turnover rates in some areas with simultaneous reduction of catecholamine levels in other hypothalamic regions. Significantly increased turnover rates for dopamine in one area of the forebrain and decreased secretion of prolactin were also observed. Commercial xylene exposure similarly resulted in significant decreases in noradrenaline levels and increased catecholamine levels accompanied by highly significant increases in catecholamine turnover rates within several areas of the hypothalamus. Significant increases in dopamine levels and turnover rates were observed in many regions of the forebrain. Prolactin secretion was not different from controls; however, TSH secretion was highly significantly depressed. These neuroendocrine changes may lead to disturbed brain function following solvent exposure (Andersson *et al.*, 1981).

Ethylbenzene Developmental Studies

CFY rats were exposed to ethylbenzene at 600, 1200 or 2400 mg/m$^3$ continuously over days 7 to 15 of gestation. All exposure levels exhibited significant retardation of fetal growth as well as statistically significant increases in resorptions and fetuses with extra ribs (Ungvary and Tatrai, 1985).

Similar exposures in New Zealand white rabbits were also evaluated. Rabbits were exposed continually to ethylbenzene at 500 or 1000 mg/m$^3$ over days 7 to 20 of gestation. There were no effects except a statistically significant decrease in mean fetal weight after the 500 mg/m$^3$ exposure. These embryotoxic effects seen in two species indicate a possibility for "spontaneous" abortions in working women (Ungvary and Tatrai, 1985).

In a review of teratogenicity of solvents, ethylbenzene was evaluated by Schardein (1993.) No evidence of teratogenicity was found in both rats and hamsters, citing a study by Hardin *et al.* in 1981.

## Xylene

The majority of articles located in this portion of the literature search indicate that xylene has endocrine disruption potential. It is important to note that xylene frequently has isomer specific effects and the relevant toxicity of isomers is dependent on the segment of the endocrine system on which it is acting. Xylene was found to cause changes in rodent brain neurotransmitter levels, similar to toluene and ethylbenzene. Xylene exposure resulted in rodent sperm abnormalities and male hormone and reproductive organ effects in some but not all studies. Developmental studies point to fetotoxicity; xylene may be a weak teratogen at very high doses. Embryotoxicity was also found *in vitro*. Many of these effects are produced at high exposure levels not found in industry. The mechanisms of action and direct endocrine action are not clear, especially in developmental studies.

### Xylene Toxicity Studies

A standard 10 day oral toxicity study in rats resulted in a significant decrease in thymus weights. Male and female Sprague-Dawley rats were gavaged daily with *o*-, *m*- or *p*-xylene in corn oil. Dose levels were 250, 1000 or 2000 mg/kg per day. The study was terminated 24 hours post-exposure. Two of the 10 female rats exposed to 2000 mg *p*-xylene/kg-day died. Absolute and relative thymus weights were decreased significantly in both males and females exposed to *p*-xylene. Additional effects included increased liver weights in males and females at both mid and high doses of each isomer, and decreased spleen weights in males at the high doses of *m*- and *o*-xylene. Males in the highest dose group of each isomer had significantly lower bodyweights as compared to controls. In the subsequent 90-day study using mixed xylenes at dose levels of 150, 750 and 1500 mg/kg per day, no thymus effects were reported (Condie *et al.*, 1988).

### Xylene Neuroendocrine Studies

Exposure of male Sprague-Dawley rats to high levels of commercial xylene and xylene isomers resulted in changes of neuroendocrine levels in parts of the brain. The rats were exposed for 6 hours per day for 3 days to 2000 ppm of either *o*-, *m*- or *p*-xylene or commercial xylene (2% *o*-, 64.5% *m*- and 10% *p*-xylene, 23% ethylbenzene and 0.5% benzene). Neuroendocrine levels were measured 16 to 18 hours post-exposure. Exposure to *o*-xylene resulted in significantly decreased dopamine turnover in several areas of the forebrain and significant increases in catecholamine levels and turnover rates in regions of the hypothalamus. Secretion of prolactin was significantly decreased. *m*-Xylene exposure caused no significant changes in forebrain dopamine levels; however, catecholamine levels and turnover rates were significantly increased in many areas of the hypothalamus. Highly significant decreases in corticosterone secretion were also observed. Similarly, *o*-xylene exposure also resulted in no significant changes of forebrain dopamine levels and highly significant changes in hypothalamic catecholamine levels and turnover rates. *p*-Xylene decreased both corticosterone and prolactin secretion. All of the

pure xylene isomers produced significant decreases in noradrenaline within some parts of the hypothalamus. Commercial xylene exposure resulted in significant decreases in noradrenaline levels and increased catecholamine levels accompanied by highly significant increases in catecholamine turnover rates within several areas of the hypothalamus. Significant increases in dopamine levels and turnover rates were observed in many regions of the forebrain. TSH secretion was depressed in a highly significant manner. These neuroendocrine changes may lead to disturbed brain function following solvent exposure (Andersson *et al.*, 1981).

The effect of xylene exposure on dopamine $D_2$ agonist binding was examined by Hillefors-Berglund *et al.* (1995). Male Sprague-Dawley rats were exposed to xylene (99% pure) at 80 ppm for 6 hours, 5 days per week for 4 weeks. The rats were observed for 26 to 32 days post-exposure and then sacrificed. Body, whole brain, caudate-putamen and subcortical limbic area weights were not significantly affected. [$^3$H]Raclopride was used to test $D_2$ agonist binding affinity. The inhibition constant, inhibition constant for low-affinity sites, inhibition constant for high-affinity sites and proportion of high-affinity sites for dopamine on [$^3$H]raclopride-binding in the caudate-putamen region were not changed as compared to air exposed control binding affinity. These parameters were also not significantly affected in the cortical limbic region. Serum prolactin levels were not different from controls at 26 to 32 days post-exposure. The Swedish TLV for xylene is 50 ppm; at 80 ppm and above, xylene was not found to increase the affinity of dopamine $D_2$ agonist binding and there was no effect on the number of $D_2$ receptors. Increased $D_2$ agonist binding affinity has been related to enhanced apomorphine-induced locomotor activity, leading to behavioral consequences.

Rank (1985) investigated female NMRI-BOM mice exposed to 1600 ppm *m*-xylene for 4 hours, 5 days per week, for 7 weeks. Mice were decapitated within 20 hours post-exposure and the amounts of [$^3$H]clonidine bound to 4 brain regions (i.e., hypothalamus, diencephalon, cortex and cerebellum) were measured. After seven weeks exposure, the specific binding of [$^3$H]clonidine to alpha-adrenergic brain receptors was significantly lower for exposed mice compared to controls in the hypothalamus region ($p<0.05$). No significant changes were observed in the other three brain regions. During the exposure the behavior of exposed mice was different from controls; the exposed mice were very active in the chamber and sweated profusely, consuming increased amounts of water and food during the exposure as a result. The controls were not active and did not consume significant quantities during exposure, but made up for this by consuming more than the exposed group between exposures. Therefore, no net difference in food consumption or bodyweights resulted. To examine if the hypothalamic effects were concurrent with the changes in food/water intake, the same experiment was repeated with only two weeks exposure. The behavioral results were similar to the seven week study; however, no significant difference was seen in the [$^3$H]clonidine specific binding in any part of the brain, including the hypothalamus. Alpha-adrenergic receptors in the hypothalamus are known to be important in control of feeding and drinking behavior. Epinephrine and dopamine also have been reported to elicit feeding and drinking responses in satiated rats. Although this study suggests that *m*-xylene disrupts the regulation of catecholamines, it is not clear whether the alteration in alpha-receptor binding in the hypothalamus region after seven weeks of *m*-xylene exposure was due to a change in receptor affinity or to a decrease in receptor density. In previous unpublished studies, Rank had found that all three xylene isomers decreased the number of alpha-adrenergic receptors. The receptor affinity was not altered by *p*-xylene and was only slightly decreased by *o*-xylene, whereas *m*-xylene increased the dissociation constant by 100%.

Xylene was found to have little adverse effect on plasma butyrylcholinesterase activity in mice. Male and female NMRI mice were continuously exposed to xylene (18% *o*-, 70% *m*-, 12% *p*-xylene and <3% ethylbenzene) at 150 ppm for 30 days. There were no significant alterations in bodyweight; however female mice had significantly increased spleen weights as compared to air exposed controls. The males showed a slight but significant increase in plasma butyrylcholinesterase activity; the authors felt this effect was not pronounced in comparison to other solvent exposures. Butyrylcholinesterase activity is regulated by the endocrine system; testosterone is mainly responsible for its activity level. Solvents such as TCE greatly increase the butyrylcholinesterase activities in male mice; a subsequent study revealed that TCE does not affect testosterone levels (i.e., synthesis and breakdown are not altered). As mice do not posses SHBG, which helps protect testosterone from breaking down in the liver, TCE is not altering this mechanism either. TCE appears to affect butyrylcholinesterase independently of testosterone and may act on its own in either the liver or the pituitary/hypothalamus. Since xylene did not affect butyrylcholinesterase activity greatly, it does not appear to decrease testosterone levels nor does it act directly in either the liver or the pituitary region on this particular enzyme (Kjellstrand *et al.*, 1985).

Xylene Reproductive Studies

Xylene combined with heat stress was found to induce sperm abnormalities in Sprague Dawley rats. Male rats were dosed intraperitoneally with 0.5 ml *ortho*-xylene/kg bodyweight at age 10 to 16 weeks. The rats were then kept at regular (20-24°C) or high (24-30°C) temperatures for five weeks. Additional rats were dosed with 1.5 ml/kg and kept at regular temperature only. The dosing vehicle was corn oil. Excessive abnormal sperm were not observed in either dose group held at regular temperatures. Significant increases in sperm abnormalities were observed in the high temperature treatment group as compared to the high temperature control group dosed only with corn oil. Abnormalities included banana-like, amorphous and excessively hooked heads as well as abnormal tail folding. Heat stress, nutritional deficiencies and some diseases are known to cause sperm abnormalities in both humans and mice. *o*-Xylene appears to act in a synergistic manner with high temperatures (Washington *et al.*, 1983).

Yamada (1993) investigated the effects of lacquer thinner and its constituents, including toluene and xylene, on the reproductive organs of male Wistar rats. Rats inhaled xylene vapor twice a day for approximately ten minutes (i.e., until the righting reflex was overcome) on seven consecutive days; the rats were sacrificed on the eighth day. Xylene significantly decreased plasma testosterone levels, prostate acid phosphatase activity or testicular and accessory organ (i.e., epididymus, vas deferens, seminal vesicles and prostate) weights. Numbers of sperm in the epididymus were also decreased. Bodyweights were not affected. Xylene appears to have interfered with testicular androgen synthesis, decreasing acid phosphatase activity and epididymal sperm counts. Acid phosphatase activity is considered an index of testosterone function and is therefore dependent on plasma testosterone levels.

In an evaluation of the testicular effects of *n*-hexane, rats were also exposed to xylene or toluene. Sprague Dawley rats inhaled xylene at 1000 ppm for 18 hours daily for 61 days. The animals were observed for up to 14 months following exposure. Rats exposed to xylene alone had no testicular alterations. Androgen synthesis, testosterone concentration, vas deferens morphology, spermatozoa morphology, noradrenaline concentration and germ cell line immunoreactivity to nerve growth factor were also not affected. Loss of immunoreactivity would

indicate a total loss of germ cells. A combined exposure of xylene and *n*-hexane was found to significantly prevent the occurrence of testicular atrophy and loss of germ cells induced by *n*-hexane alone (Nylen *et al.*, 1989).

## Xylene Developmental Studies

### Pre-Mating/Gestational Exposure

Male and female Charles River rats were exposed to mixed xylenes in a 1992 study by Bio/Dynamics, Inc. Exposure to 60, 250 or 500 ppm lasted 6 hours daily for 131 days prior to mating and throughout a 20 day mating period. Females continued exposure through day 20 of gestation. On day 21 of gestation, mating indices were found to be significantly lower than controls in the mid and high dose groups. At the high dose, significantly increased resorptions and decreased female fetus weights were found. Male fetus weights were not significantly different from controls.

### Gestational Exposure

A NOAEL of 10 mg/m$^3$ was reported for white Wistar rats exposed during gestation. Dams were exposed to 10, 50 or 500 mg/m$^3$ mixed toluene isomers, 6 hours daily, on gestation days 1 through 21. The mid and high dose groups had increased post-implantation losses, growth retardation of fetuses, including delayed skull and sternebrae ossification, and anomalies of organs (e.g., hydrocephalus, microphthalmia, intracerebral hematomas and liver hemorrhages) (Mirkova *et al.*, 1983). As hematomas and hemorrhages are not typically classed as teratogenic effects and as the relative number of true teratogenic outcomes (i.e., hydrocephalus and microphthalmia) was not given, the teratogenic capability of xylene under these conditions is ambiguous (Hood and Ottley, 1985).

CFY rats exposed to a xylene mixture during gestation showed embryotoxicity, but not teratogenicity, in the 1978 Hudak and Ungvary study. Rats continuously exposed to 1000 mg/m$^3$ (230 ppm) xylene on days 9 through 14 of gestation had embryos with significantly increased occurrence of skeletal anomalies including fused sternebrae and extra ribs. This exposure level was not maternally lethal.

In 1980, Ungvary *et al.* examined embryotoxicity of the individual xylene isomers. CFY rats were exposed continuously to 150, 1500 or 3000 mg/m$^3$ (35, 350 or 700 ppm) of analytical grade *o*-, *m*- or *p*-xylene on days 7 through 14 of gestation. Fetal effects were analyzed on day 21 of gestation. Each isomer was maternally toxic. *m*-Xylene was maternally lethal in 4 of 30 dams. *m*- and *p*-xylenes significantly decreased food intake during the high dose exposure; *o*-xylene lowered food intake at both the mid and high doses. Maternal weight gain was decreased in the *m*-xylene high exposure group as compared to controls. Relative liver weights were significantly increased at all *o*-xylene dose levels. Additionally, each caused significant fetal development retardation including decreased weight and increased slowness of skeletal development at the highest dose levels. Fetal weight was also decreased at the mid *o*-xylene dose; signs of skeletal retardation were significantly increased at all *p*-xylene dose levels. Decreased enzyme, succinic dehydrogenase, acid and alkaline phosphatase, and glucose 6-

phosphate activities were noted; these activities are indicators of nephron maturity. Fetal effects were isomer and dose dependent; the relative severity of fetal retardation caused by the isomers was *para-* > *ortho-* > *meta-*. The highest dose of *p*-xylene decreased mean litter size, induced the appearance of extra ribs and increased preimplantation and postimplantation fetal losses. *o*-Xylene also induced extra ribs and preimplantation fetal loss at the high dose. Separate groups of dams were exposed to 150, 1500 or 3000 mg/m$^3$ of *o*-xylene for only 2 hours on day 18 of gestation. Immediately following exposure, maternal blood xylene concentrations were found to be proportional to the atmospheric concentrations. Xylene was found in greater concentrations in fetal blood than amniotic fluid; concentration in both were lower than maternal blood levels.

In a later study, Ungvary *et al.* (1981) found indications of endocrine disruption caused by *p*-xylene in CFY dams. Rats were exposed continually to 3000 mg/m$^3$ *p*-xylene on day 10 or days 9 and 10 of gestation; effects parameters were measured on day 11 (i.e., 2 hours after exposure termination). Mean fetal weight was significantly decreased in the 48 hour exposure group as compared to controls. Progesterone and 17β-estradiol levels in peripheral blood (uterine and femoral veins) were also decreased in 48 hour exposed dams. Uterine and ovarian venous outflow volumes were not disturbed and secretion rates of hormones were not affected by exposure. *p*-Xylene apparently induces the hepatic monooxygenase system responsible for initiating metabolism of progesterone and 17β-estradiol. Decreased circulating levels of these hormones may assist in causing the embryotoxic (fetal growth retardation and lethality) effects seen in other studies.

*p*-Xylene exposure *in utero* had previously been found to have embryotoxic effects in a 1979 study by Ungvary *et al.* Rats were exposed to 150, 1500 or 3000 mg/m$^3$ *p*-xylene continuously on days 7 through 14 of gestation. Maternal lethality or toxicity was not found at these levels. However, increased placental weights, increased pre-implantation losses and skeletal retardation were present in toluene exposed pups. At the high dose, fetal weight gain was also decreased and the occurrence of extra ribs increased. Decreases in the number of enzyme-active nephrons and the activities of succinic dehydrogenase, alkaline, acidic and glucose-6-phosphatase also occurred at the high dose.

A mixed xylene study reviewed by Hood and Ottley (1985) provided some evidence of fetotoxicity. Rats were exposed to 435 or 1739 mg/m$^3$ mixed xylene (i.e., 36.1, 11.4, 52.1, 0.31 and 0.12% ethylbenzene, *o*-, *m*-, *p*-xylene and toluene, respectively). Maternal toxicity, fetal malformations and fetal weight effects were not seen. At the high dose, an insignificant increase in resorptions was noted. However, a significant increase in retarded skeletal ossification was observed at the high dose, although the majority of affected fetuses came from only three litters (25 litters examined) (Litton Bionetics, 1978).

In the same review paper (Hood and Ottley, 1985), a dermal teratogenicity study was reported. Rats were treated with 100, 200 or 2000 mg/kg per day on gestation days 1 through 20. Maternal brain levels of cholinesterase and cytochrome oxidase activities were decreased at all dose levels. Maternal glucose-6-phosphate dehydrogenase activity was decreased at the mid and high dose levels. Open field activity of dams was significantly decreased at the high dose while emotionality (i.e., number of defecations) increased. Fetal brain cholinesterase and cytochrome oxidase activities were also decreased at the mid and high dose groups; maleate, iso-citrate and glucose-6-phosphate dehydrogenase activities increased. Fetal behavioral tests were not performed (Mirkova *et al.*, 1979).

In a 1993 dual teratogenicity/postnatal study by Hass and Jakobsen, Wistar rats were exposed to technical grade xylene at 500 ppm for 6 hours per day over days 4 to 20 of gestation. The technical grade of xylene contains all isomers, but is predominantly *m*-xylene; technical xylene may also contain up to 35% ethylbenzene. No maternal effects were seen during these studies. For the teratology study, pups were examined on day 21 of gestation. A small but not significant increase in preimplantation loss was seen in exposed rats. However, a highly significant ($p<0.001$) increased incidence of delayed ossification of the *os maxillare* was found in the exposed litters (18/26) as compared to control litters (2/22). In the postnatal portion, pups were born naturally and monitored for 28 days. Birth weights and pup weights at 28 days were significantly higher in the exposed group as opposed to controls. Pups were tested with a Rotarod on days 22 through 24. Females in the exposed group stayed on the Rotarod for significantly shorter times as compared to control females on days 22 and 23. Treated males had significantly short times only on day 23.

A later study by Hass *et al.* (1995) further examined the effects of xylene on postnatal development in Wistar rats. Dams were exposed to 500 ppm technical grade xylene (15% ethylbenzene, 19% *o*-, 45% *m*- and 20% *p*-xylene) for 6 hours per day over days 7 through 20 of gestation. Two males and two females per litter were weaned at 22 days and kept for behavioral testing. No maternal toxicity was observed in this study. Although no significant changes were found in fetal bodyweights, significantly low absolute brain weights were found in exposed animals; relative brain weights were not different from controls. The air righting reflex was significantly delayed in exposed animals. Closer examination comparing male and female offspring revealed that females were significantly delayed on postnatal days 15 and 16 while male offspring were not significantly delayed as compared to male controls; retesting on day 17 did not reveal differences in reflexes. This delay may indicate damage to vestibular function. Although exposed offspring had lower scores on the Rotarod neuromotor abilities test and female offspring were more affected than male, these differences were not significant. Open field activity tests also did not detect significant differences between exposed and control rats. During the Morris water maze learning and memory test performed at three months of age, exposed animals took slightly, but not significantly, longer during the initial learning (submerged platform in one quadrant) and reversal learning stages (platform in opposite quadrant). There was a significant increase in times for exposed animals to find the platform when relocated to the center of the maze; again, exposed females used significantly more time than exposed males or control females. Increased Morris maze times have been linked to hippocampal dysfunction in adult rats.

A postnatal study of rats exposed *in utero* to *p*-xylene alone showed no evidence of developmental toxicity. Sprague Dawley dams were exposed to 3500 or 7000 mg/m$^3$ *p*-xylene 6 hours per day on days 7 through 16 of gestation. Litters were normalized on postnatal day 4 and weaned on postnatal day 21. The high exposure dams had significantly decreased weight gain as compared with controls. Litter size and pup weights were not significantly affected. CNS development was measured with acoustic startle response and figure-8 maze tests. Motor activity measured on postnatal days 22 and 65 in the Figure-8 maze did not differ significantly between control and exposed groups. Acoustic startle response measured on days 13, 17, 21 or 63 were also not significantly different from controls in latency, amplitude or sensitization. *p*-Xylene was not developmentally toxic at even maternally toxic levels in this study (Rosen *et al.*, 1986).

In a multiple species study, CFY rats exposed to xylene for 2 hours at 200, 2500 or 5000 mg/m$^3$ on the 18th or 20th day of gestation were found to have xylene blood concentrations proportional to the exposure level. Lower concentrations of xylene were found in fetal blood, which had higher xylene concentrations than amniotic fluid. Rats exposed at 250, 1900 or 3400 mg/m$^3$ continuously over days 7 to 15 of gestation had significantly increased incidence of growth retarded fetuses. At the highest exposure level, one dam died and there were statistically significant increases in resorptions and fetuses with extra ribs (Ungvary and Tatrai, 1985).

In the same study, CFLP mice were exposed to 500 or 1000 mg/m$^3$ commercial xylene for 3 four-hour sessions daily on days 6 through 15 of gestation, resulting in significant increases in growth retarded fetuses. Mice were also exposed under the same regimen to 500 mg/m$^3$ of *o*-, *m*- or *p*-xylene. Each isomer produced significantly increased fetal retardation (Ungvary and Tatrai, 1985).

Similar exposures in New Zealand white rabbits were also evaluated. Rabbits exposed continually to commercial xylene at 500 or 1000 mg/m$^3$ over days 7 to 20 of gestation responded with significantly decreased mean fetal weight. The higher exposure also led to a significant increase in percent relative liver weight in the dams. Rabbits were also exposed to 500 or 1000 mg/m$^3$ *o*-, *m*- or *p*-xylene for the same duration. There were no effects except a statistically significant increase in resorbed fetuses after the 500 mg/m$^3$ *m*-xylene exposure and the death of a dam in the 1000 mg/m$^3$ *p*-xylene study group. These embryotoxic effects seen in three species indicate a possibility for "spontaneous" abortions in working women (Ungvary and Tatrai, 1985).

In a review of the teratogenicity of solvents, Schardein (1993) found mixed results for xylene. Isomers *m*-, *o*- and *p*-xylene were each found to be non-teratogenic in rats (Tatrai *et al.*, 1979; Hudak *et al.*, 1980; Krotov and Chebotar, 1972). However, when the isomers were tested individually by Nawrot and Staples (1981), each was found to be teratogenic in mice. The Krotov and Chebotar study was mentioned in another review by Hood and Ottley (1985). Rats were exposed to *p*-xylene at 500 mg/m$^3$ throughout gestation. An apparent increase in pre- and post-implantation losses was seen in treated dams, although statistical methods were vague. No teratogenicity was found. Similarly, the Nawrot and Staples study was reviewed by Hood and Ottley. Mice were gavaged 3 times daily with individual isomers of xylene at 780, 1960 or 2610 mg/kg per day during different stages of gestation. *m*-Xylene exposure resulted in maternal toxicity and increased resorptions at the highest dose when dosed on days 6 through 15 of gestation. The high dose of *m*-xylene given on days 12 through 15 resulted in increased prenatal deaths and increased cleft palate malformations. During another trial with *m*-xylene on days 6 through 15, clefting was again increased. *o*-Xylene exposure on days 6 through 15 resulted in maternal toxicity, increased resorptions and cleft palates at the mid and high doses. Given on days 12 through 15, only prenatal deaths were increased as compared to controls. *p*-Xylene exposure on days 6 through 15 similarly caused toxicity, prenatal death and cleft palate at the mid and high doses. Exposure on days 12 through 15 resulted in increased prenatal death and increased incidence of cleft palate at the high dose. The cleft palate malformations were not thought to be caused by growth retardation but appeared to be a weak teratogenic response.

Embryotoxicity and teratogenicity were found in a 1982 Marks *et al.* study using albino CD-1 mice. Mice received commercial xylene (17.0% ethylbenzene, 60.2% *m*-, 9.1% *o*- and 13.6%

*p*-xylene) by gavage in cottonseed oil 3 times daily over days 6 through 15 of gestation. Daily dose levels were 0.6, 1.2, 2.4, 3.0, 3.6 or 4.8 ml/kg bodyweight (i.e., 0.52, 1.03, 2.06, 2.58, 3.10 or 4.13 mg/kg-day). Fetuses and dams were examined on day 18. The NOAEL was 1.2 ml/kg-day. At 2.4 ml/kg-day, significantly increased maternal liver weights and decreased average fetal weights were observed. A significant increase in fetal malformations occurred; malformations included cleft palate and open eye. Effects increased in a dose dependent manner at 3.0 ml/kg; wavy rib malformations (bilateral and multiple) were also found at this dosage. The 3.6 ml/kg dose was lethal to 12 of 38 dams and caused significant decreases in maternal weight gain. The percentage of resorptions was also significantly increased at this dosage. The highest dosage level was universally lethal.

A mouse inhalation toxicity study was featured in the Hood and Ottley review (1985). Shigeta *et al.* (1983) exposed dams to 500, 1000, 2000 or 4000 ppm xylene of unknown composition for 6 hours daily during gestation days 6 through 12. Fetal weights in the 2000 and 4000 ppm groups were significantly decreased. Although no malformations were found, a dose-response relationship for altered rib numbers and delayed ossification of sternebrae was established. Xylene exposed pups allowed to develop showed delays in hair coat growth and lower weight gain as compared to control pups.

Xylene *In Vitro*/Screening Studies

An *in vitro* study using 10.5 day old rat embryos incubated in xylene solutions for 40 hours found a dose dependent increase in embryotoxicity. The NOEL solution concentration was 1.08 μmol/ml. At 1.89 μmol/ml, embryo growth, as measured by crown-rump length and protein content of the embryos, was significantly decreased as compared to controls. Development, as measured by somite numbers, was also significantly decreased. Growth and development, including decreased yolk sac diameter, were more impaired at 2.70 μmol/ml, in a dose-response manner. Severe growth retardation effects included decreases of more than two standard deviations in the size of the telencephalon as compared to embryos of the same crown-rump length (Brown-Woodman *et al.*, 1994b). The telencephalon includes the cerebrum, basal ganglia and the limbic system (McCance and Huether, 1990). Embryos incubated in xylene for only the first 16 hours of the 40 hour culture period showed similar growth, development and yolk sac decreases, including a greater decrease in protein content than the 40 hour embryos. A mixture of toluene and xylene together produced additive effects in embryos incubated for 40 hours. *In vitro* incubation concentrations are relative to serum concentrations. The published maximum permissible occupational blood concentration of xylene is 0.028 μmol/ml, allowing a safety factor of 40 over the NOEL in this study. However, embryos exposed to xylene at different times of gestation or throughout gestation may have a different NOEL (Brown-Woodman *et al.*, 1994b).

A previous, similar study by the same researchers did not produce a NOEL. Sprague-Dawley rat embryos were explanted at 9.5 days and incubated in 0.1, 0.5 or 1.0 μl/ml (initial concentration) commercial xylene (containing all xylene isomers and ethylbenzene) for 48 hours. At 0.1 μl/ml, crown-rump length was significantly decreased as compared to control embryos. Exposure at 0.5 μl/ml resulted in significantly decreased yolk sac diameter and somite number as well as crown-rump length. These growth and developmental parameters decreased in a dose dependent manner with the highest concentration exposure. The

industrial maximum permissible blood level for xylene is 0.003 µl/ml, providing a safety margin before the studied embryotoxic effects would likely occur (Brown-Woodman *et al.*, 1991).

Xylenes were found to have low A/D (adult/developmental) minimal effective concentration ratios when evaluated in the hydra developmental assay. Xylene isomers *m*-, *o*- and *p*- were found to have ratios of 1.0, 1.5 and 1.5, respectively. Mixed xylenes gave a ratio of 2.0. Therefore, xylenes disrupt development only at or near concentrations that are toxic to adults (Johnson *et al.*, 1986). These were comparable to A/D ratios calculated from published mammalian studies (A/D of 1 for all isomers and mixtures). Ratios less than 3 are generally not differentiated from each other; priority for further testing (i.e., mammalian testing) goes to chemicals with ratios greater than 3 or those greater than 1 with widespread human usage and potential impact (Johnson *et al.*, 1988).

## RESEARCH NEEDS AND RECOMMENDATIONS

Chemicals are being developed at a much more rapid pace than our ability to test their toxicity. The experimental data listed in this paper indicate a number of toxicant-induced disruptions of endocrine functions. Several of the studies do not address mechanisms by which normal physiologic events go awry. This is particularly evident in reproductive toxicology, where it is necessary to investigate the multifactorial events of pregnancy and the effects of xenobiotic exposure for several generations. Although multigenerational studies and chronic toxicity tests may screen out potential toxins, little information is gained on the modes of action. Closer scrutiny of xenobiotic effects on the critical events in reproduction are warranted. With this understanding, better management and use of xenobiotics can be expected.

At present, monitoring is inconsistently performed in workplaces and other environments that are considered to impose risks to reproduction or other physiological functions influenced by hormones. It is not clear how much endocrine disruption will be shown to be due to such environmental exposures; however, it is evident that we will need to monitor for such toxicity if we hope to identify it when it occurs. One of the limitations in that effort is the inherent variability in normal endocrine functions. For example there is much variability in normal measures of reproductive competence, such as menstrual cyclicity and semen analysis parameters. Research needs to characterize the normal range and interindividual reproducibility of reproductive endpoints.

Epidemiological data have proven useful for the identification of human carcinogens. Occupational clinicians should work with toxicologists to become educated regarding links between workplace exposures and clinical effects. Medical records, including death certificates, often lack any detailed information regarding patients' exposure. Detailed exposure information could improve the significance of mortality and incidence. Mortality studies on breast cancer can be useful for establishing social and economic relationships to health, but often provide few meaningful clues for identifying potential environmental toxins which may induce cancer through endocrine disruption. Incidence studies are better suited; however, without detailed health histories and exposure information, meaningful analysis from medical records cannot be performed. This is unfortunate because death certificates and medical records represent an inexpensive source of chronic dose response information. It is important is to identify, register and follow populations with documented and quantitatively verified exposures.

Another problem with human epidemiological studies in identifying reproductive outcome of exposure to a xenobiotic is that only the health of the exposed individuals, not the offspring, were questioned. It is not surprising that many human studies have failed to link adverse health effects with exposure to xenobiotics. The lesson learned from wildlife reveals the importance of considering the health of the offspring of the exposed individuals. For example, when seeking causal links for loss of fertility or immune competency among cohorts, the subjects' prenatal and early postnatal exposure must be considered (i.e., what were the parents' exposures?). Prenatal and perinatal exposure to xenobiotics probably has more influence on fertility than any other exposure throughout a lifetime, due to sensitive periods of sexual differentiation (Olsen and Skoc, 1993).

Much information exists in the files of regulatory authorities and the agrochemical industry concerning the reproductive toxicity of currently used pesticides. The majority of this information is not available in the open published literature. Efforts should be made to ensure that this material is made available.

Monitoring wildlife populations provides a source of hypothesis generation regarding harmful factors in the environment. In fact, wildlife population surveys are attributed with bringing the effects of EDC's to the attention of the scientific community. Monitoring wildlife is difficult, requiring years of observation to avoid confusion with natural fluctuations in population density. Therefore, efforts should made to: be more aware of and develop protocols to assess functional damage in the field; test in the laboratory the hypotheses generated in the field; find early markers in developing tissue that predict long-term delayed effects on functionality of both wildlife and humans; test the hypothesis that there are links between cell differentiation during development and cancer; and break disciplinary boundaries and collaborate with those responsible for public health, policy and risk communication.

The primary exposure pathway of humans to endocrine-like substances is through the diet. It includes both natural products and contaminants (e.g., pesticides) which bind to diverse receptors including estrogen, progesterone, androgen, vitamin D, retinoic acid and Ah receptors. Available data on dietary human intakes and serum levels suggest that for some endocrine-like substances, namely estrogen and Ah receptor agonists, the naturally occurring phytoestrogens are present in significantly higher concentrations than synthetic agonists from industrial sources (Safe and Gaido, 1997). The overall health and reproductive impacts of these substances require additional data on individual compounds and mixtures as well as their interactive effects within and between various endocrine response pathways. Efforts should be made to quantify exposures to both naturally occurring and synthetic endocrine-like substances in our diet.

Biomarkers of endocrine disruption, such as vitellogenin induction in male fish from exposure to estrogenic substances, are needed as screening tools for exposure assessment, as biological indices of latent effects and as means to address mechanistic issues related to identifying critical steps in the process or to understand the basis for species differences in response. Multigenerational studies are needed to identify biomarkers in offspring that can be measured shortly after exposure and that are predictive of long-term effects. In the case of ecological biomarkers, field evaluations are needed to establish which early changes or endpoints in individuals are the most predictive of population-level effects. Available human and wildlife tissues need to be measured for the presence of EDCs to compare with levels in the food chain (Kavlock *et al.,* 1996).

In recent years much promising work has been done with 3-D Quantitative Structure Activity Relationships (QSAR) models. Such models are being developed for ligand receptor interactions or $EC_{50}$ data derived from androgen, estrogen and progesterone competitive binding assays (Kavlock *et al.,* 1996). These data are being used as a training set to develop the QSAR models. The developed models are then tested with chemicals of known activity and, when validated, the models can be used to screen libraries of compounds with unknown activities. Similar approaches have been successful for Ah receptor-ligands. Research is needed to validate and expand the training sets of these models for steroids and for other hormone-receptor interactions. Models could also be developed for toxicant-enzyme interactions. False negatives can occur using this technique for chemicals with molecular structures different from those in the training set; however, as more chemicals are validated, the precision of this technique will improve.

Databases should be developed containing occupational or medical exposure data which can be made available to researchers. Wildlife databases could be created that contain data on population sizes, habitats, reproductive rates or surveillance data on occurrences of tumors or deformations. Such databases could provide the basis for establishing time related trends.

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# APPENDIX A:
# CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| 2,4-D | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| 2,4,5-T | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| 2,8-dibenzylcyclo-octanone | | | | reported to be estrogenic | | | McLachlan JA *et al*., 1984, Rundam Appl Toxicol 4:686-91 |
| 3,9-dihydroxybenz[a]-anthracene | potential metabolite of benz[a] anthracene | | | reported to be estrogenic | | | McLachlan JA & Newbold RR, 1987, Environ Health Perspect 75:25-7 |
| 4-nonylphenol | | | | E-screen assay result indicates full agonist at 1 umol/L in human serum | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-96 |
| 4-octylphenol | | | | E-screen assay result indicates full agonist at 100 nmol/L in human serum | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-95 |
| Alachlor | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Aldicarb | nematocide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| alkyl phenols | non-biodegradable detergents, antioxidants from polystyrenes & PVCs | | | | | | RA Monitor, 28 May 96 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| alkylphenol (polystyrene-derived) | surfactants & ind. detergents | 50 mg | | endometrial mitotic index higher than ovariectomized rats & lower than one E2 rat | ovariectomized adult Sprague-Dawley female rats primed with 15 ng E2 | sc in sesame oil | Soto A *et al.*, 1991, Environ Health Perspect 92:167-74 |
| Amitrole | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Aroclor 1221 | | | | enhanced proliferative potency | human breast E sensitive MCF7 cells | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| atrazine | weed killer | | | proven xenoestrogen | | | Davis DL & Bradlow HL, 1995, Sci Am 273(4)167-70, 172 |
| Atrazine | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| atrazine | | | | incr. hormone release | | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| atrazine | | 750 ppm | | incr. mam. tumor | M rat | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| Benomyl | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| benzo(a)pyrene | | | | inhibit estrogen pathway I, induces mam. tumors | | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| benzo(a)pyrene | | | | mam. tumors | | GI | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| benzylbutyl-phthalate | | | | E-screen assay result indicates partial agonist at 10 umol/L in human serum | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-98 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| bisphenol-a | autoclaved polycarbonate plastic flasks | | | "estrogenicity" | | | Cotton P, 1994, JAMA, 271(6):414, 416 |
| bisphenol-a | | | | E-screen assay result indicates partial agonist at 1 umol/L in human serum | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-99 |
| bisphenol-a dimethacrylate | | | | E-screen assay result indicates partial agonist at 1 umol/L | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-100 |
| butylhydroxy-anisole (tert-) | | | | E-screen assay result indicates full agonist at 50 umol/L in human serum | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-97 |
| cadmium | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| carbaryl | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| chlordane | termite killer | | | proven xenoestrogen | | | Davis DL & Bradlow HL, 1995, Sci Am 273(4)167-70, 172 |
| chlordane | termite killer | | | enhanced proliferative potency | human breast E sensitive MCF7 cells | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| chlordane | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| chlordane | pesticide | ND - conc too low for response | IC50 inhibitory conc. of hER binding | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| chlordane | pesticide | ND - conc too low for response | EC50 (conc to acheive 50% *B*-Galactosidace activity | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| chlordecone | | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| DBCP | nematocide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| DDT (*o,p'*) | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| DDT (technical grade) | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| DDT & metabolites | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| DDT & products (*o,p'* DDT) | | | | initiated implantation & maintained pregnancy, uterotropic (incr. weight), inhibits binding of [3H]estradiol to uterine cytosolic E receptor | rat | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| DDT & products (*o,p'* DDT) | | | | accelerated mam. tumors (mice treated with 2-acetamidophenanthrene) | M mice | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| DEHP (di(2-ethylhexyl)-phthalate) | | | | | | | RA Monitor, 28 May 96 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| desmethyl-tamoxifen (n-) | | | | E-screen assay result indicates partial agonist | | | Sonnenschein C *et al.*, 1985, Life Sci 37(4):387-94 |
| dicofol | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| dieldrin | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| dieldrin | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| dieldrin | pesticide | | | indicates estrogenicity by induction of PS2 & PGR in MCF7 cells at 1 umol/L | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-103 |
| dieldrin | pesticide | >50 (uM) | IC50 inhibitory conc. of hER binding | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| dieldrin | pesticide | >33 (uM) | EC50 (conc to acheive 50% *B*-Galactosidace activity | | | | Arnold SF et al., 1996 |
| dimethyl benzanthracene (DMBA) | | | | inhibit estrogen pathway I, induces mam. tumors | | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| dioxin (2,3,7,8-TCDD) | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| endosulfan | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| endosulfan | pesticide | | | indicates estrogenicity by induction of PS2 & PGR in MCF7 cells at 1 pmol/L | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-101 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| endosulfan (alpha) | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| endosulfan (beta) | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| endosulfan (technical grade) | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| endosulfan | pesticide | >33 (uM) | EC50 (conc to acheive 50% *B*-Galactosidace activity | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| estradiol | | .001 (uM) | IC50 inhibitory conc.of hER binding | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| estradiol | | .0001 (uM) | EC50 (conc to acheive 50% *B*-Galactosidace activity | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| furans (2,3,7,8,-TCDF) | | | | | | | RA Monitor, 28 May 96 |
| HCH (beta-) | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| heptachlor | | | | enhanced proliferative potency | human breast E sensitive MCF7 cells | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| heptachlor & H-epoxide | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| hexachloro-benzene | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| hexachloro-cyclohexane (*B*-) | | | | induces cystosolic P receptor, redistributes E receptors | | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| hexachloro-cyclohexane congeners (lindane) | | | | | | | RA Monitor, 28 May 96 |
| Kelthane | | | | | | | RA Monitor, 28 May 96 |
| Kepone (chlordecone) | | | | initiated implantation & maintained pregnancy | rat | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| Kepone (chlordecone) | | | | enhanced proliferative potency | human breast E sensitive MCF7 cells | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| lab animal & pet food products | | | | | | | RA Monitor, 28 May 96 |
| lead | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| lindane (gamma-HCH) | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Mancozeb | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Maneb | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| mercury | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Methomyl | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| methoxychlor | insecticide | | | proven xenoestrogen | | | Davis DL & Bradlow HL, 1995, Sci Am 273(4)167-70, 172 |
| methoxychlor | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| methoxychlor | | | | initiated implantation & maintained pregnancy | rat | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| Metiram-complex | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Metribuzin | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Mirex | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Nitrofen | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| nonyphenol | antioxidant for manufacturing plastic; in detergents, toiletries, lubricants & spermicides | | | "estrogenicity" | | | Cotton P, 1994, JAMA, 271(6):414, 416 |
| nonylphenol (ind. grade (approx. 85% p-nonylphenol)) | plastic additive | | | induced cell proliferation, progesterone receptors in human MCF7 breast tumor cells & mitotic activity in rat endometrium | | | Soto A *et al.*, 1991, Environ Health Perspect 92:167-73 |
| octachloro-styrene | | | | | | | RA Monitor, 28 May 96 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| organo-tin compounds (tributylin) | | | | | | | RA Monitor, 28 May 96 |
| Oxychlordane | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| PAHs (methyl-cholanthrene) | | | | | | | Davis *et al.*, 1995 |
| Parathion | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| PBBs | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| PCBs | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| PCBs | electrical transformer fluids | | | proven xenoestrogen | | | Davis DL & Bradlow HL, 1995, Sci Am 273(4)167-70, 172 |
| PCBs | | | | E receptors bind to probes | | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| PCBs (hydroxilated, various congeners) | | | | Studied to determine C50 for Er binding. Increased binding affinity for PCBs with ortho chlorine substitutions. Mice injected with ortho congeners resulted in increased uterine weight vs. nonortho congeners. | | | Korach KD *et al.*, 1987, Molec Pharmacol 33:120-6 |
| pentachloro-phenol (PCP) | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| penta- to nonylphenols | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| phthalates | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| soy products | | | | | | | RA Monitor, 28 May 96 |
| styrene dimers & trimers | | | | | | | RA Monitor, 28 May 96 |
| styrenes | | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| synthetic pyrethroids | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| tamoxiphen | | | | E-screen assay result indicates partial agonist | | | Sonnenschein C *et al.*, 1985, Life Sci 37(4):387-94 |
| tamoxiphen | triphenyl-ethylene antiestrogen | | | monohydroxy metabolite has greatly enhanced affinity for E receptor compared to parent compound | | | McLachlan JA *et al*., 1984, Fundam Appl Toxicol 4:686-91 |
| tamoxiphen (cis-) | | | | E-screen assay result indicates total agonist | | | Sonnenschein C *et al.*, 1985, Life Sci 37(4):387-94 |
| toxaphene | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| toxaphene | insecticide | | | E-screen assay result indicates partial agonist at 10 uM in human serum | | | Soto AM *et al.*, 1994, Environ Health Persect 102(4):380-3 |
| toxaphene | pesticide | | | indicates estrogenicity by induction of PS2 & PGR in MCF7 cells at 1 umol/L | | | Sonnenschein A *et al.*, 1995, Clin Chem 41(12):1888-102 |

APPENDIX A:
CHEMICALS IDENTIFIED BY SEARCHES AS ENDOCRINE DISRUPTING

| Chemical or Mixture | Chemical Use | Dose | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|
| toxaphene | pesticide | >50 (uM) 470 uM was reported by Soto et al. 1994 | IC50 inhibitory conc. of hER binding | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| toxaphene | pesticide | >33 (uM) | EC50 (conc to acheive 50% *B*-Galactosidace activity | | yeast estrogen system (YES) containing (hER) | | Arnold SF et al., 1996 |
| Transnonachlor | insecticide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| tributyl tin | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Trifluralin | herbicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Zineb | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| Ziram | fungicide | | | reported to have reprod and ED effects | | | Colburn T *et al.*, 1993, Environ Health Perspect 101(5):378-84 |
| | | | | | | | |
| | Cell proliferation assays such as E-screen are more sensitive than those that induce gene products | | | | | | |
| | (i.e., estrogen dose needed to induce maximal cell proliferation is ~50-100 times lower than that needed to elicit | | | | | | |
| | max induction of PGR or PS2) (Sonnenschein *et al.*, 1995). | | | | | | |

# APPENDIX B:
# MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| 1,1-Dibromoethane | | 0-100 ppm | | estimated high dose to induce mam. gland cancer | | rat, mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-9 |
| 1,1-Dichloroethane | solvent | >500 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-9 |
| 1,2,3-Trichloropropane | solvent | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-10 |
| 1,2-Dibromo-3-chloropropane | soil fumigant (nematocide) | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-11 |
| 1,2-Dibromo-3-chloropropane | soil fumigant (nematocide) | 100-500 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-11 |
| 1,2-Dibromoethane | soil fumigant, lead scavenger | | | | | | | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-12 |
| 1,2-Dichloroethane | fumigant/ insecticide intermediate | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-13 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| 1,1-Dibromoethane | | M rat | F rat | M mus | F mus | |
| 1,1-Dichloroethane | | n | y/n | n | n | |
| 1,2,3-Trichloropropane | | y | y | y | y | |
| 1,2-Dibromo-3-chloropropane | | y | y | | | |
| 1,2-Dibromo-3-chloropropane | | | | y | y | |
| 1,2-Dibromoethane | | y | y | y | y | |
| 1,2-Dichloroethane | | y | y | | | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| 1,2-Dichloroethane | fumigant/ insecticide intermediate | 100-500 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-13 |
| 1,2-Dichloropropane | chemical intermediate, dry cleaning solvent, fumigant | 100-500 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-14 |
| 1,3-Butadiene | rubber manufacturing | 100-700 ppm | | estimated high dose to induce mam. gland cancer | | mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-15 |
| 1,8-Dinitropyrene | urban air, food, combustion | 16 uM total (3 x/wk) | 4 wk | | 42% incidence mam. tumors | weanling F SD rats | ip | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-90 |
| 1,8-Dinitropyrene | urban air; food, combustion | 16 uM total | | | 61% incidence mam. tumors | weanling F SD rats | ig | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-90 |
| 1-Nitropyrene | urban air, food, combustion | 800 uM total (1 x/wk) | 16 wk | | 63% incidence mam. tumors | weanling F SD rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 1-Nitropyrene | urban air, food, combustion | 250 uM total (1 x/wk) | 8 wk | | 31% incidence mam. tumors | weanling F SD rats | sc | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 1-Nitropyrene | urban air, food, combustion | 16 uM total (3 x/wk) | 4 wk | | 30% incidence mam. tumors | weanling F SD rats | ip | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| 1,2-Dichloroethane | | | | y | y | |
| 1,2-Dichloropropane | | n | y/n | y | y | |
| 1,3-Butadiene | | | | y | y | |
| 1,8-Dinitropyrene | | | y | | | |
| 1,8-Dinitropyrene | | | y | | | |
| 1-Nitropyrene | | | y | | | |
| 1-Nitropyrene | | | y | | | |
| 1-Nitropyrene | | | y | | | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| 1-Nitropyrene | urban air, food, combustion | 16 uM total | | | 46% incidence mam. tumors | weanling F SD rats | ig | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 2,3-Dibromo-1-propanol | flame retardant | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | dermal | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-14 |
| 2,4-2,6-Toluene diisocyanate | flexible polyurethane foam manufacturing | | | | | | | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-15 |
| 2,4-Diaminotoluene | dye intermediate | | | | | | | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-16 |
| 2,4-Dinitrotoluene | dye intermediate | 10-100 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-17 |
| 2-(Acetylamino) fluorene | synthetic | 0.3-0.5 mM total | | | 30% incidence mam. tumors | 6 wks-old F SD rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 2-Chloroaceto-phenone | flame retardant | 0-100 ppm | | estimated high dose to induce mam. gland cancer | | rat, mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-17 |
| 2-Nitropyrene | | | | | | | | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| 1-Nitropyrene | | | y | | | |
| 2,3-Dibromo-1-propanol | | y | y | y | y | |
| 2,4-2,6-Toluene diisocyanate | | y | y | n | y | |
| 2,4-Diaminotoluene | | y | y | n | y | |
| 2,4-Dinitrotoluene | | y | y | n | n | |
| 2-(Acetylamino) fluorene | | | y | | | |
| 2-Chloroaceto-phenone | | n | y/n | n | n | |
| 2-Nitropyrene | | | | | | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| 3,3'-Dimethoxybenzidine dihydrochloride | dye intermediate | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | dr. H2O | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-17 |
| 3,3'-Dimethylbenzidine dihydrochloride | dye intermediate | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | dr. H2O | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| 4-Nitropyrene | urban air | 12 mM total | | | 82% incidence mam. tumors | weanling F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 5-Nitroacenaphthene | research chemical | 100-500 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| 7,12-Dimethylbenz(a) anthracene | synthetic | 8 uM total | | | 100% incidence in mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 7,12-Dimethylbenz(a) anthracene | synthetic | 2 uM total | | | 17% incidence in mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 7,12-Dimethylbenz(a) anthracene | synthetic | 3.9 uM total | | | 10% incidence in mam. tumors | 8 wks-old F SD rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| 7,12-Dimethylbenz(a) anthracene | synthetic | 78 uM total | | | 100% incidence in mam. tumors | 8 wks-old F SD rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| 3,3'-Dimethoxybenzidine dihydrochloride | | y | y | | | |
| 3,3'-Dimethylbenzidine dihydrochloride | | y | y | | | |
| 4-Nitropyrene | | | y | | | |
| 5-Nitroacenaphthene | | y | y | n | y | |
| 7,12-Dimethylbenz(a) anthracene | | | y | | | |
| 7,12-Dimethylbenz(a) anthracene | | | y | | | |
| 7,12-Dimethylbenz(a) anthracene | | | y | | | |
| 7,12-Dimethylbenz(a) anthracene | | | y | | | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| 7,12-Dimethylbenz(a) anthracene | synthetic | 19.5 uM total | | | 88% incidence in mam. tumors | 8 wks-old F SD rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Benzene | solvent | 100-500 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Benzene | | | | | mam. cancer | | oral, ihl | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| Benzo(a)pyrene | urban air, food, combustion | 8 uM total | | | 0% incidence mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Benzo(a)pyrene | urban air, food, combustion | 2 uM total | | | 5% incidence mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Benzo(a)pyrene | urban air, food, combustion | 200 uM total (8 doses) | | | 67% incidence mam. tumors | 50 dy-old inbred virgin F LEW/MAI rats | oral | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Butadiene | | | | | | | | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Carbon tetrachloride | | | | | | | | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| 7,12-Dimethylbenz(a) anthracene | | | y | | | |
| Benzene | | y | y | y | y | |
| Benzene | | y | y | y | y | |
| Benzo(a)pyrene | | | y | | | |
| Benzo(a)pyrene | | | y | | | |
| Benzo(a)pyrene | | | y | | | |
| Butadiene | | | | | | |
| Carbon tetrachloride | | | | | | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| Clonitralid | molluscicide | 10-100 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| dibenz(ah) anthracene | | | | | mam. tumors | | GI | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |
| Dibenzo(a,l)pyrene | urban air | 8 uM total | | | 100% incidence in mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Dibenzo(a,l)pyrene | urban air | 2 uM total | | | 100% incidence in mam. tumors | 8 wks-old F SD rats | intra-mam. injection | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Dichlorourethane | | | | | | | | el-Bayoumy K, 1992, Chem Res Toxicol 5(5):585-91 |
| Dichlorvos | pesticide | 0.1-10 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Dichlorvos | pesticide | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Ethylene oxide | chemical intermediate | 100-700 ppm | | estimated high dose to induce mam. gland cancer | | mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| Clonitralid | | n | y/n | | n | |
| dibenz(ah) anthracene | | | | | | |
| Dibenzo(a,l)pyrene | | | y | | | |
| Dibenzo(a,l)pyrene | | | y | | | |
| Dichlorourethane | | | | | | |
| Dichlorvos | | y | y/n | | | |
| Dichlorvos | | | | y | y | |
| Ethylene oxide | | | | y | y | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| Glycidol | vinyl polymer stabilizer | 10-100 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat, mice | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Hydrazobenzene | dye intermediate | 10-100 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Methylene chloride (dichloromethane) | solvent | 2000-4000 ppm | | estimated high dose to induce mam. gland cancer | | rat, mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Nithiazide | antiprotozoal compound | 100-500 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Ochratoxin A | mycotoxin | 0.1-10 mg/kg-dy | | estimated high dose to induce mam. gland cancer | | rat | oral | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Sulfallate | herbicide | 100-700 ppm | | estimated high dose to induce mam. gland cancer | | rat | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| Sulfallate | herbicide | 700-2000 ppm | | estimated high dose to induce mam. gland cancer | | mice | ihl | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| Glycidol | | y | y | y | y | |
| Hydrazobenzene | | y | y | n | y | |
| Methylene chloride (dichloromethane) | | y | y | y | y | |
| Nithiazide | | n | y | y | y | |
| Ochratoxin A | | y | y | | | |
| Sulfallate | | y | y | | | |
| Sulfallate | | | | y | y | |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Chemical Use | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| | | | | | | | | |
| Toluidine hydrochloride (o-) | dye intermediate | 100-500 mg/kg-dy | 2 yr | estimated high dose to induce mam. gland cancer | | rat, mice | feed | Dunnick JK *et al.*, 1995, Carcinogenesis 16(2):173-18 |
| triazines | | 750 ppm | 126 weeks | | incr. mam. tumor | M rat | | Davis DL *et al.*, 1993, Environ Health Perspect 101(5):372-7 |

APPENDIX B:
MAMMARY CARCINOGENS IDENTIFIED IN EDC SEARCHES

| Chemical or Mixture | Mammary neoplasms in rodents (y=some/clear evidence, n=no/equivocal evidence, y/n=equivocal evidence) | | | | | |
|---|---|---|---|---|---|---|
| | | | | | | |
| Toluidine hydrochloride (*o*-) | | y | y | y | y | |
| triazines | | | | | | |

# APPENDIX C:
# REPRODUCTIVE DISRUPTORS IDENTIFIED IN EDC SEARCHES

APPENDIX C:
REPRODUCTIVE DISRUPTORS IDENTIFIED IN EDC SEARCHES

| Chemical | Dose | Duration | Response Level (ECx or LDx?) | Response | Species | Route | Reference | Comments |
|---|---|---|---|---|---|---|---|---|
| 1,2-Dibromo-3-chloropropane | 5.4 mg/kg-dy (100 ppm) | 64 dy | NOAEL | | M SD rats, 8 10 wks-old | dr. H2O | Heindel JJ *et al.*, 1989, Fundam Appl Toxicol 13:804-16 | Sperm effects seen in other studies cited. |
| 1,2-Dibromo-3-chloropropane | 9.7 mg/kg-dy (200 ppm) | 64 dy | LOAEL | apparently not gonadotoxic, decr. absolute testicular wt., decr. BW gain, decr. H2O consumption | M SD rats, 8 10 wks-old | dr. H2O | Heindel JJ *et al.*, 1989, Fundam Appl Toxicol 13:804-16 | Sperm effects seen in other studies cited. |